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# (12) United States Patent

Cutler et al.

(54) CONTROL OF PLANT STRESS TOLERANCE, WATER USE EFFICIENCY AND GENE EXPRESSION USING NOVEL ABA RECEPTOR PROTEINS AND SYNTHETIC AGONISTS

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	C12N 9/16	(2006.01)
	C07K 14/415	(2006.01)
	A01N 37/08	(2006.01)
	A01N 37/10	(2006.01)
	A01N 37/42	(2006.01)
	A01N 41/06	(2006.01)
	A01N 43/10	(2006.01)
	A01N 43/40	(2006.01)
	A01N 43/42	(2006.01)
	A01N 43/50	(2006.01)
	A01N 43/54	(2006.01)
	A01N 43/82	(2006.01)
	A01N 47/30	(2006.01)
	A01N 37/06	(2006.01)
	A01N 37/28	(2006.01)
	A01N 37/34	(2006.01)
	A01N 43/713	(2006.01)
(52)	II C CI	

(52) U.S. Cl.

# (10) Patent No.: US 9,315,821 B2

(45) **Date of Patent:** Apr. 19, 2016

# (58) Field of Classification Search

None

See application file for complete search history.

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# (57) ABSTRACT

The present invention provides methods of regulating plant stress tolerance.

# 23 Claims, 24 Drawing Sheets

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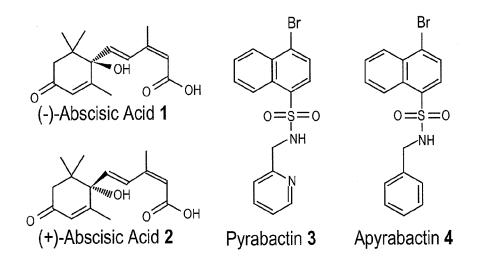
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# FIG. 1A

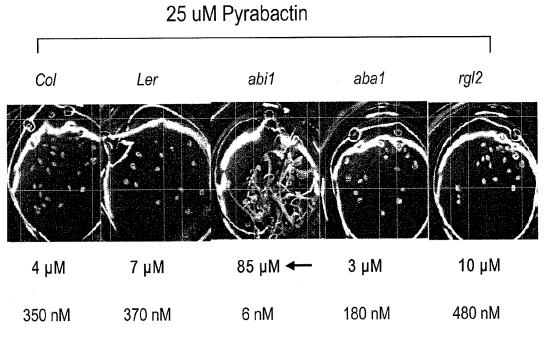
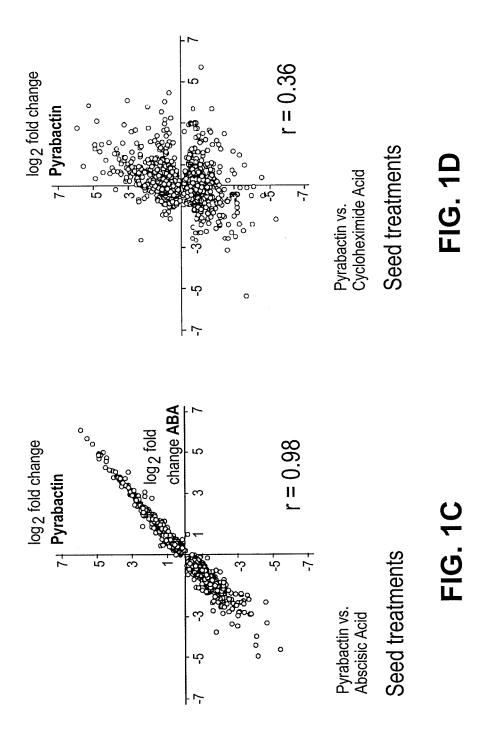
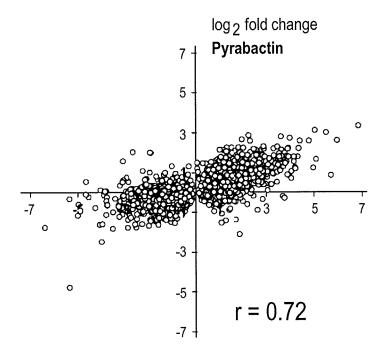


FIG. 1B





Pyrabactin vs. Abscisic Acid

Seedling treatments

FIG. 1E

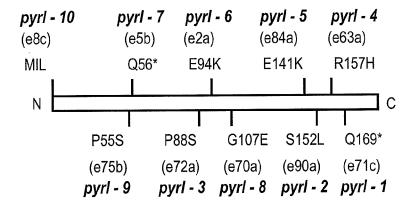
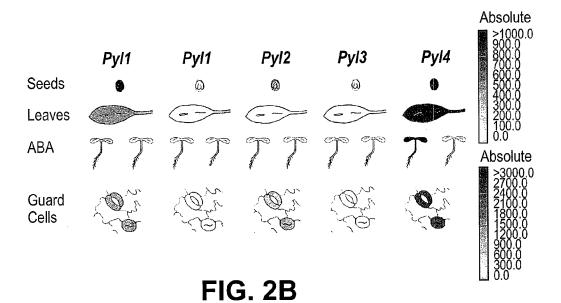
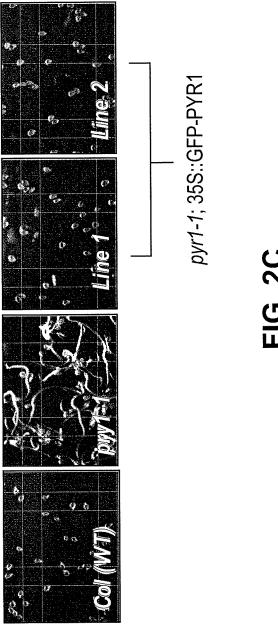
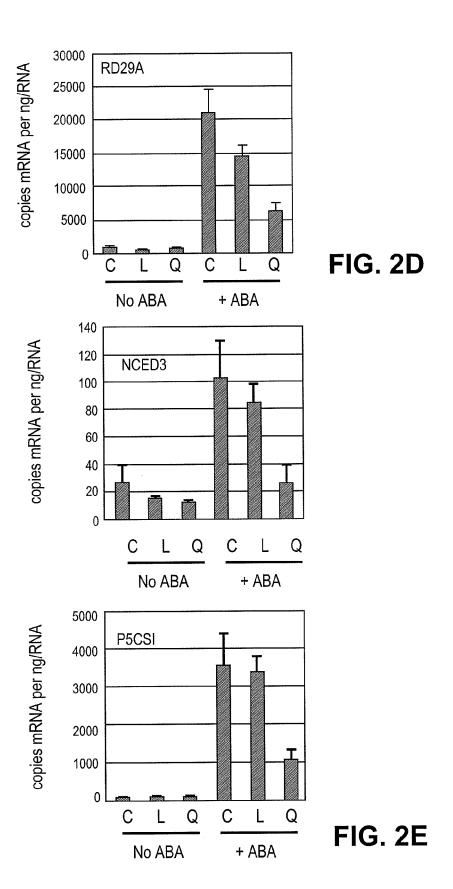
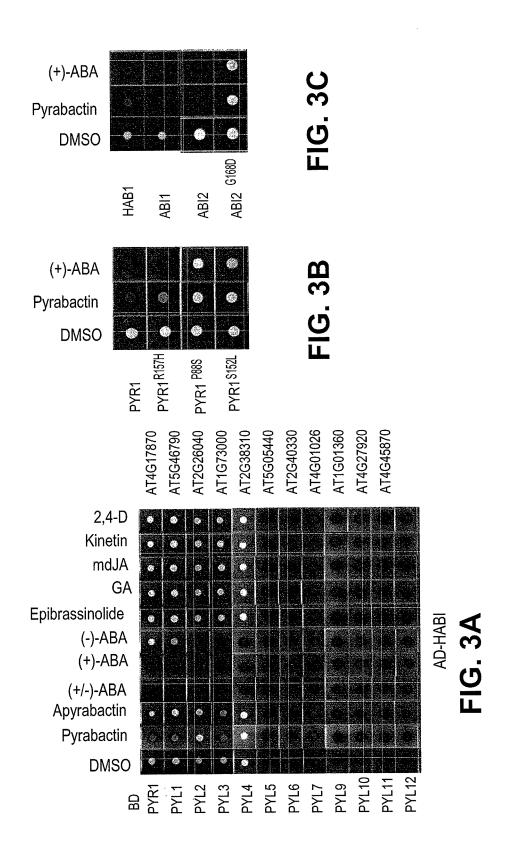


FIG. 2A









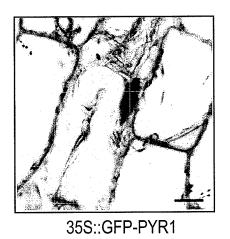


FIG. 4

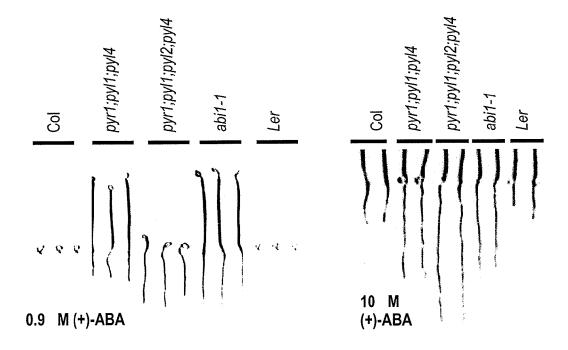


FIG. 5A

FIG. 5B

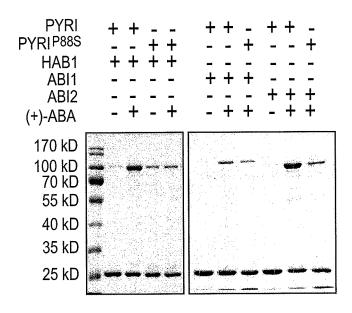


FIG. 6A

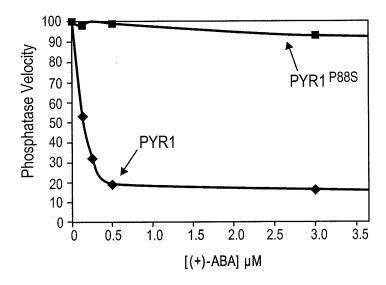


FIG. 6B

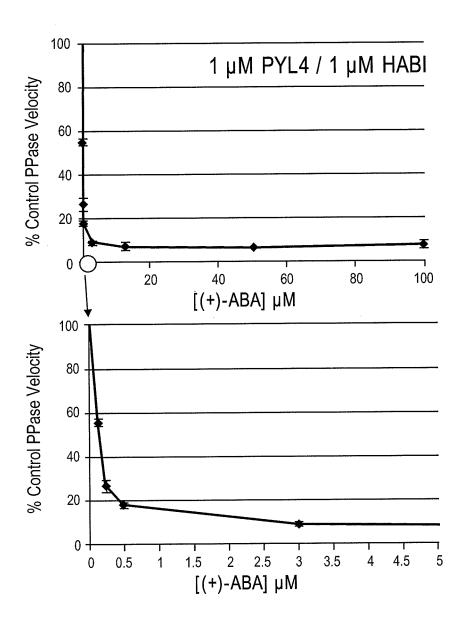
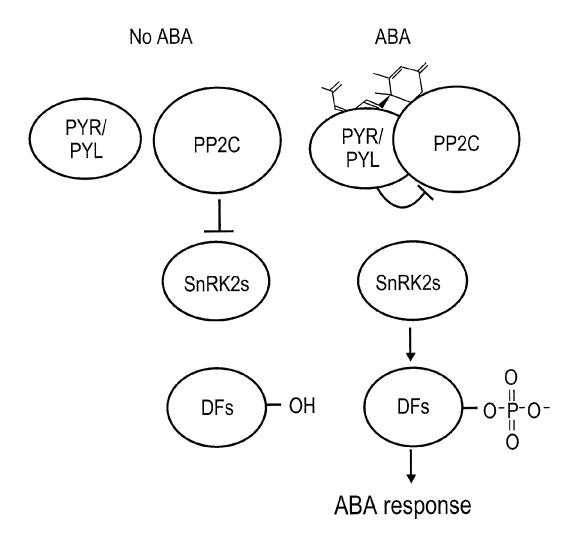


FIG. 6C



**FIG.** 7

		S	trong Red	ceptor Re Compour	esponde i	to
Entry	Compound	PYR1	PYRL1	PYL2	PYL3	PYL4
1	(+)-Abscisic Acid	+	+	-	+	+
2	Pyrabactin	+	+		+	
3	HO HN N F	_			+	+
4	Br S O CH3		+	_	+	+
5	0° 0		+	_	_	
6	0-N+ S O O O O O O O O O O O O O O O O O O	+	+	_		+

FIG. 8

		S	trong Red	ceptor Ro Compoui	esponde nd	to
Entry	Compound	PYR1	PYRL1	PYL2	PYL3	PYL4
7	#7563159 F F	+	+			
8	F O S H N S		_		+	
9	H <sub>3</sub> C CH <sub>3</sub> O N N N  HN S O F  #7561035		<del></del>	_	+	_
10				_	+	

FIG. 8 (cont.)

		S	trong Red	ceptor Re Compou	esponde nd	to
Entry	Compound	PYR1		PYL2	PYL3	PYL4
11	0=s=0 N N S		_	_	+	
12	CH <sub>3</sub> HN O	_		_	+	+
13	CH <sub>3</sub> O NH  S O CH <sub>3</sub>	_	_	_	+	+
14	F F O S S O O CH3		_	_	+	
15	CI FF F NH H <sub>3</sub> C		_		+	+

FIG. 8 (cont.)

		S	trong Red	ceptor Re Compoui	esponde nd	to
Entry	Compound	PYR1	PYRL1	PYL2	PYL3	PYL4
16	0 = S = 0 $0 = S = 0$ $0 = S = 0$				+	
17	-O. N. O. N.		<u>.</u>		+	_
18	H <sub>3</sub> C N S F F F		_	+	+	+
19	O HN		_	+	+	+

FIG. 8 (cont.)

		Recepto	or	
Compound	PYR1	PYL1	PYL2	PYL3
(+)-ABA	0.2	0.2	0.1	0.1
Pyrabactin	0.4	0.6	>50	>50
7653159	0.5	0.55	>50	>50
6655097	0.4	0.5	>50	>50
7561035	>50	>50	4	5

 $IC_{50}$  values are shown in  $\mu M$ 

Apr. 19, 2016

Measured using PYR/PYL protein indicated (i.e. receptor) and the PP2C HAB1 with the phophatase substrate pNPP

FIG. 9

Trait	Control	PYL4-OE	Quadruple lof
Flowering time	++	+	+++
Stature	+++	++	+
Chlorophyll content	+	++	+
Wiltiness	++	+	++++

Flowers early, small flowers late, dark plant, very wilty green, less wilty

Quadruple = pyr1/pyl1/pyl2/pyl quadruple mutant

**PYL4-OE** = transgenic Arabidopsis plants with Rbcs promotor driving GFP-PYL4, Rbcs = subisco small subunit (high expression promoter).

FIG. 10

# FIG. 11A

Alignment

PYR1	LKNSIAEFHTY 23	
PVL1	MANSESSSSPVNEEENSQRISTLHHQTMPSDLTQDEFTQLSQSIAEFHTY 50	
PYL2	LEPVIKTYHQF 28	
PYL3	MNLAPIHDPSSSSTTTTSSSTPYGLTKDEFSTLDSIIRTHHTF 43	
PYL4	MLAVHRPSSAVSDG-DSVQIPMMIASFQKRFPSLSRDSTAARFHTH 45	
PYL5	MRSPVQLQHGSDATNGF-HTLQPHDQTDGPIKRV-CLTRGMH-VPEHVAMHHTH 51	
PYL6	MPTSIQFQRSSTAAEAANATVRNYPHHHQKQVQKVSLTRGMADVPEHVELSHTH 54	
PYL7	MEMIGGDDTDTEMYGALVTAQSLRLRHLH 29	
PYL9	MMDGVEGGTAMYGGLETVQYVRTHHQH 27	
PYL10	SEYIKKHHRH 20	
PYL8	MEANGIENLTNPNQEREFIRRHKH 25	
PYL11		
PYL12	8	
PYL13		
ALL_Con		
1_12_con	Taract	
1_6_ Con	AXA	
7_10_Con	НХН	

# FIG. 11B

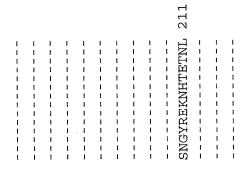
PYR1	OLDPGSCSSLHAQRIHAPPELVWSIVRRFDKPQTYKHFIKSCSVEQNFEMRVGCT 78	
PYL1	QLGNGRCSSLLAQRIHAPPETVWSVVRRFDRPQIYKHFIKSCNVSEDFEMRVGCT 105	
PYL2	EPDPTTCTSLITQRIHAPASVVWPLIRRFDNPERYKHFVKRCRL-ISGDGDVGSV 82	
PYL3	PRSPNTCTSLIAHRVDAPAHAIWRFVRDFANPNKYKHFIKSCTIRVNGNGIKE-IKVGTI 102	٥1
PYL4	EVGPNQCCSAVIQEISAPISTVWSVVRRFDNPQAYKHFLKSCSVIGGDGDNVGSL 100	_
PYL5	DVGPDQCCSSVVQMIHAPPESVWALVRRFDNPKVYKNFIRQCRIVQGDGLHVGDL 106	
PYL6	VVGPSQCFSVVVQDVEAPVSTVWSILSRFEHPQAYKHFVKSCHVVIGDGREVGSV 109	Φ.
PYL7	HCRENQCTSVLVKYIQAPVHLVWSLVRRFDQPQKYKPFISRCTVNG-DPEIGCL 82	
PYL9	LCRENQCTSALVKHIKAPLHLVWSLVRRFDQPQKYKPFVSRCTVIG-DPEIGSL 80	
PYL10	ELVESQCSSTLVKHIKAPLHLVWSIVRRFDEPQKYKPFISRCVVQGKKLEVGSV 74	
PYL8	ELVDNQCSSTLVKHINAPVHIVWSLVRRFDQPQKYKPFISRCVVKG-NMEIGTV 78	
PYL11	SQKYHTCGSTLVQTIDAPLSLVWSILRRFDNPQAYKQFVKTCNLSSGDGGEGSV 57	
PYL12	SQEQHVCGSTVVQTINAPLPLVWSILRRFDNPKTFKHFVKTCKLRSGDGGEGSV 57	
PYL13	S-KOKRCRSSVVETIEAPLPLVWSILRSFDKPQAYORFVKSCTMRSGGGGGKGGEGKGSV 62	
	······································	
ALL Con	CXSXXXXXXAPXXXXFXXPXXXXFXXXC	
$1 \frac{1}{2}$ Con	CXSXXXXXXAPXXXXWXXXXFXXPXXXKXFXXXC	
1_6 _Con	XXXXXXCXSXXXXXXAPXXXXWXXXXXFXXPXXYKXFXXXC	
$7\overline{10}$ Con	XXXXXQCXSXLVKXIXAPXHXVWSXVRRFDXPQKYKPFXSRCXVXGX	
11_13_Con	CXSXXVXTIXAPLXLVWSILRXFDxPXXXXXFVKXCXXXSGXGG GSV	

# FIG. 11C

PYR1	RDVIVISGLPANTSTERLDILDDERRVTGFSIIGGEHRLTNYKSVTTVHRFEKEN 133
PYL1	
PYL2	REVIVISGLPASISIERLEFVDDDHRVLSFRVVGGEHRLKNYKSVTSVNEFLNQDSGK 140
PYL3	REVSVVSGLPASTSVEILEVLDEEKRILSFRVLGGEHRLNNYRSVTSVNEFVVLEKDKKK 162
PYL4	RQVHVVSGLPAASSTERLDILDDERHVISFSVVGGDHRLSNYRSVTTLHPSPISG 155
PYL5	REVMVVSGLPAVSSTERLEILDEERHVISFSVVGGDHRLKNYRSVTTLHASDDEG 161
PYL6	REVRVVSGLPAAFSLERLEIMDDDRHVISFSVVGGDHRLMNYKSVTTVHESEEDSDGK 167
PYL7	: :
PYL9	 
PYL10	REVDLKSGLPATKSTEVLEILDDNEHILGIRIVGGDHRLKNYSSTISLHSETIDGK 130
PYL8	REVDVKSGLPATRSTERLELLDDNEHILSIRIVGGDHRLKNYSSIISLHPETIEGR 134
PYL11	REVIVVSGLPAEFSRERLDELDDESHVMMISIIGGDHRLVNYRSKTMAFVAADTEE 113
PYL12	REVTVVSDLPASFSLERLDELDDESHVMVISIIGGDHRLVNYQSKTTVFVAAE-EE 112
PYL13	RDVTLVSGFPADFSTERLEELDDESHVMVVSIIGGNHRLVNYKSKTKVVASPE 115
	* ** *****
ALL Con	RXVXXXSXXPAXXSXEXLXXXD GGXHKLXNYXS
1 12 Con	RXVXXXSXLPAXXSXEXLXXXD GGXHRLXNYXS
1_6 Con	RXVXVXSGLPAXXSXEXLXXXDXXXXXXXFXXXGGXHRLXNYXSVT
$7\overline{10}$ Con	REVXXKSGLPATXSTEXLEXLDDXEHILXIXIXGGDHRLKNYSSXXXXHXEXIXGX
11_13_Con	${ t RxVTxVSxxPAxFSxERLxELDDESHVMxxSIIGGxHRLVNYxSKT}$

191	221	190	209	207	203	215	197	187	183	188	161	159	119					
RIWTVVLESYVVDMPEGNSEDDTRMFADTVVKLNLQKLATVAEAMARNSGDGSGSQVT	RIWTVVLESYVVDVPEGNSEEDTRLFADTVIRLNLQKLASITEAMNRNNNNNSSQVR	-VYTVVLESYTVDIPEGNTEEDTKMFVDTVVKLNLQKLGVAATSAPMHDDE	RVYSVVLESYIVDIPQGNTEEDTRMFVDTVVKSNLQNLAVISTASPT	TVVVESYVVDVPPGNTKEETCDFVDVIVRCNLQSLAKIAENTAAESKKKMSL	TVVVESYIVDVPPGNTEEETLSFVDTIVRCNLQSLARSTNRQ	-KRTRVVESYVVDVPAGNDKEETCSFADTIVRCNLQSLAKLAENTSKFS	-SGTMVMESFVVDVPQGNTKDDTCYFVESLIKCNLKSLACVSERLAAQDITNSIATFCNA	-AGTMVIESFVVDVPQGNTKDETCYFVEALIRCNLKSLADVSERLASQDITQ	-TGTLAIESFVVDVPEGNTKEETCFFVEALIQCNLNSLADVTERLQAES-MEKKI	-IGTLVIESFVVDVPEGNTKDETCYFVEALIKCNLKSLADISERLAVQDTTESRV	KTVVVESYVVDVPEGNSEETTSFADTIVGFNLKSLAKLSERVAHLKL	KTVVVESYVVDVPEGNTEEETTLFADTIVGCNLRSLAKLSEKMMELT			ESXXVDXPXGNXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXX	${\tt VxESYxVDxPxGNxxxxTxxFxDxxxxxNLQxL}$	$\mathtt{xGTxxxESFVVDVPxGNTKxxTCxFVExLIxCNLxSLAxxxERL}$	
PYR1	PYL1	PYL2	PYL3	PYL4	PYL5	PYL6	PYL7	PYL9	PYL10	PYL8	PYL11	PYL12	PYL13	AI.L. Con	1 12 Con	1_6_Con	7_10_con	11_13_Con

# FIG. 11E



PYR1
PYL1
PYL2
PYL2
PYL4
PYL5
PYL6
PYL11
PYL11
PYL12
PYL12
PYL13
PYL17
PYL10
PYL10
PYL10

COMPOUND	PYR1	PYL2	PYL3	PYL4
HO	ND	+	+++	+++
ОН	-	-	++	++
H O OH	-	-	++	++
HOO	-	-	+	+

FIG. 12

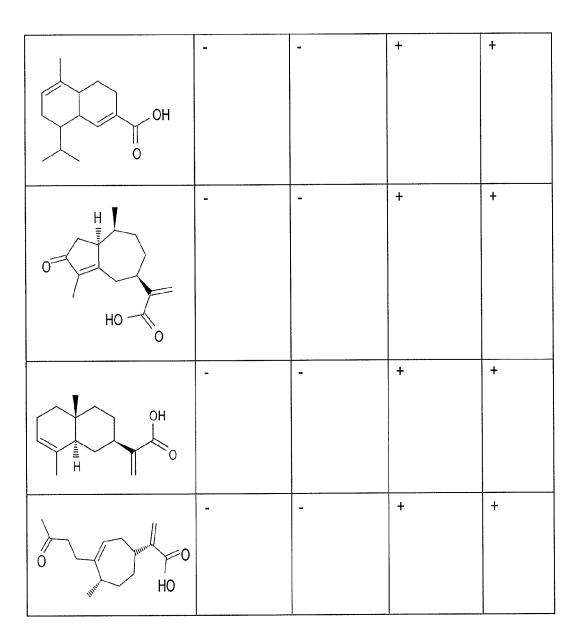


FIG. 12 (cont.)

# CONTROL OF PLANT STRESS TOLERANCE, WATER USE EFFICIENCY AND GENE EXPRESSION USING NOVEL ABA RECEPTOR PROTEINS AND SYNTHETIC AGONISTS

# CROSS-REFERENCE TO RELATED PATENT APPLICATIONS

The present patent application claims benefit of priority to U.S. Provisional Patent Application No. 61/207,684, filed Feb. 13, 2009, which is incorporated by reference for all purposes.

# BACKGROUND OF THE INVENTION

Abscisic acid (ABA) has been the focus of intense investigation since it was identified in the 1960s as an endogenous small molecule growth inhibitor and regulator of plant stress physiology (K. Ohkuma, J. L. Lyon, F. T. Addicott, O. E. Smith, Science 142, 1592 (1963); C. F. Eagles, P. E. Wareing, 20 Physiologia Plantarum 17, 697 (1964); J. W. Cornforth, B. V. Milborrow, G. Ryback, Nature 206, 715 (1965); J. W. Cornforth, B. V. Milborrow, G. Ryback, P. F. Wareing, Nature 205, 1269 (1965); D. Imber, M. Tal, Science 169 592 (1970)). Indeed, when one increases plant ABA sensitivity, improved 25 drought and other stress tolerance results. See, e.g. Wang et al., Plant J. 43:413-424 (2005); Pei et al. Science 282:287-290 (1998); US Patent Publication No 2004/0010821. Genetic analyses have identified many factors involved in ABA signaling, including the type 2 C protein phosphatases 30 (PP2Cs) ABI1, ABI2 and relatives that form the closely related ABI1/AHG1 clades that function as redundant negative regulators of ABA signaling (R. R. Finkelstein, S. S. L. Gampala, C. D. Rock, The Plant Cell 14, S15 (2002); P. McCourt, Annual Review of Plant Physiology and Plant 35 Molecular Biology 50, 219 (1999); A. Schweighofer, H. Hirt, I. Meskiene, Trends in Plant Science 9, 236 (2004)). Several ABA binding proteins have been reported, however it is not clear how they regulate the myriad effects of ABA, because they do not appear to act through known regulators of its 40 signaling pathway (X. Liu et al., Science 315, 1712 (Mar. 23, 2007); F. A. Razem, A. El-Kereamy, S. R. Abrams, R. D. Hill, Nature 439, 290 (2006); Y. Y. Shen et al., Nature 443, 823 (Oct. 19, 2006)). Additionally, the characterized receptors show negligible binding to the non-natural stereoisomer (-)- 45 ABA 1 at concentrations ~1000-fold higher than their K<sub>a</sub>s for (+)-ABA 2. (-)-ABA is bioactive in most ABA assays (B.-L. Lin, H.-J. Wang, J.-S. Wang, L. I. Zaharia, S. R. Abrams, Journal of Experimental Botany 56, 2935 (2005); D. Huang et al., The Plant Journal 50, 414 (2007)) and acts through the 50 same signaling pathway as (+)-ABA (E. Nambara et al., Genetics 161, 1247 (July, 2002)), suggesting that receptors that recognize both (-) and (+)-ABA remain to be discovered.

# BRIEF SUMMARY OF THE INVENTION

The present invention provides for plants (or a plant cell, seed, flower, leaf, fruit, or other plant part from such plants) comprising a heterologous expression cassette, the expression cassette comprising a promoter operably linked to a 60 polynucleotide encoding a PYR/PYL receptor polypeptide, wherein the plant has improved stress tolerance compared to a plant lacking the expression cassette.

In some embodiments, the PYR/PYL receptor polypeptide comprises one or more of SEQ ID NOs:1, 91, 92, 93, 94, 95, 96, 97, 98, 99, 100, 101, 102, 103, 104, 105, 106, 107 and/or 138.

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In some embodiments, the PYR/PYL receptor polypeptide is at least 70% (e.g., at least 70%, 80%, 90%, 95%) identical to any of SEO ID NOs:2-90 or 108-137.

In some embodiments, the PYR/PYL receptor polypeptide is a constitutively-active form such that the receptor will bind a type 2 protein phosphatase (PP2C) in a yeast two-hybrid assay in the absence of abscisic acid or an ABA agonist.

In some embodiments, the PYR/PYL receptor polypeptide bind a type 2 protein phosphatase (PP2C) in a yeast two-hybrid assay in the presence, but not in the absence, of abscisic acid or an ABA agonist.

In some embodiments, the plant has improved drought tolerance compared to a plant lacking the expression cassette.

In some embodiments, the promoter is a root-specific promoter

In some embodiments, the promoter is specific for an aerial portion of the plant.

In some embodiments, the promoter is inducible.

The present invention also provides for methods of increasing stress tolerance in a plant as described above. In some embodiments, the method comprises contacting the plant with a sufficient amount of a compound to increase stress tolerance compared to not contacting the plant with the compound, wherein the compound is selected from the following formulas:

$$R^{1} - \stackrel{O}{\underset{\longrightarrow}{\parallel}} - \stackrel{(CH_{2})_{n}}{\underset{\longrightarrow}{-}} - R^{2}$$

$$\begin{array}{c} O \\ R^4-S-CH_2 \end{array} \begin{array}{c} O \\ X-R^5 \end{array}$$

$$\mathbb{R}^{6} - \mathbb{N} \stackrel{\mathrm{O}}{\longrightarrow} \mathbb{C} \mathbb{N}$$

$$R^{7}-Y \xrightarrow{O} N - Z - R^{8}$$

$$R^{9}$$
(IV)

$$(\mathbb{R}^{10})_{p} \underbrace{\hspace{1cm}}_{(\mathbb{R}^{11})_{r}}$$

$$\mathbb{R}^{12} \xrightarrow{\mathbb{R}^{14}} \mathbb{R}^{13}$$
(VI)

wherein

55

 $\rm R^1$  is selected from the group consisting of aryl and heteroaryl, optionally substituted with 1-3  $\rm R^{1\it a}$  groups; each  $\rm R^{1\it a}$  is independently selected from the group consisting of H, halogen,  $\rm C_{1-6}$  alkyl,  $\rm C_{1-6}$  haloalkyl,  $\rm C_{2-6}$  alkenyl,  $\rm C_{2-6}$  alkynyl,  $\rm C_{1-6}$  alkoxy,  $\rm C_{1-6}$  haloalkoxy,  $\rm C_{1-6}$ 

 $\begin{array}{l} \mbox{hydroxyalkyl}, -\mbox{NR'R"}, -\mbox{SR'}, -\mbox{OH}, -\mbox{CN}, -\mbox{NO}_2, \\ -\mbox{C(O)R'}, -\mbox{C(O)OR'}, -\mbox{C(O)NR'R"}, -\mbox{N(R')C(O)} \\ \mbox{R"}, -\mbox{N(R')C(O)OR"}, -\mbox{N(R')C(O)NR'R"}, -\mbox{OP(O)} \\ \mbox{(OR')}_2, -\mbox{S(O)}_2\mbox{OR'}, -\mbox{S(O)}_2\mbox{NR'R"}, \mbox{cycloalkyl}, \mbox{heterocycloalkyl}, \mbox{ard the heteroaryl group is optionally substituted with } -\mbox{NO}_2 \mbox{ and the heteroaryl group is optionally substituted with $C_{1-6}$ alkyl;} \end{array}$ 

alternatively, adjacent R<sup>1a</sup> groups can combine to form a member selected from the group consisting of cycloalkyl, heterocycloalkyl, aryl and heteroaryl, 10 wherein the aryl group is optionally substituted with —OH;

R' and R" are each independently selected from the group consisting of H and  $\rm C_{1-6}$  alkyl;

 $R^2$  is selected from the group consisting of  $C_{2-6}$  alkenyl, 15 cycloalkenyl, aryl and heteroaryl;

R<sup>3</sup> is H or is optionally combined with R<sup>2</sup> and the atoms to which each is attached to form a heterocycloalkyl optionally substituted with 1-3 R<sup>1a</sup> groups;

R<sup>4</sup> is a heteroaryl, optionally substituted with 1-3 R<sup>1a</sup> 20 groups;

 $R^5$  is selected from the group consisting of  $C_{1-6}$  alkyl and aryl, wherein the aryl is optionally substituted with 1-3  $R^{1a}$  groups;

each of R<sup>6</sup> and R<sup>7</sup> are independently selected from the 25 group consisting of aryl and heteroaryl, each optionally substituted with 1-3 R<sup>1</sup> groups;

R<sup>8</sup> is selected from the group consisting of cycloalkyl and aryl, each optionally substituted with 1-3 R<sup>1α</sup> groups;

R<sup>9</sup> is H or is optionally combined with a R<sup>1a</sup> group of R<sup>8</sup> 30 and the atoms to which each is attached to form a heterocycloalkyl; subscript n is 0-2;

X is absent or is selected from the group consisting of —O—, and —N(R')—;

Y is absent or is selected from the group consisting of 35 —C(O)— and —C(R',R")—;

Z is absent or is selected from the group consisting of —N=, and —C(S)—N(R')—, such that one of Y and Z is absent;

each of  $R^{10}$  and  $R^{11}$  are independently selected from the  $\,$  40 group consisting of H, C  $_{1\text{-}6}$  alkyl, —C(O)OR', and C  $_{1\text{-}6}$  alkenyl-C(O)OH, wherein at least two of the  $R^{10}$  and  $R^{11}$  groups are  $C_{1\text{-}6}$  alkyl and at least one of the  $R^{10}$  and  $R^{11}$  groups is  $C_{1\text{-}6}$  alkenyl-C(O)OH;

alternatively, two R<sup>10</sup> or R<sup>11</sup> groups attached to the same 45 carbon are combined to form =O;

alternatively, one R<sup>10</sup> group and one R<sup>11</sup> group are combined to form a cycloalkyl having from 3 to 6 ring members;

each of subscripts k and m is an integer from 1 to 3, such 50 that the sum of k and m is from 3 to 4;

each of subscripts p and r is an integer from 1 to 10;

wherein two of the R<sup>10</sup> and R<sup>11</sup> groups on adjacent carbons are combined to form a bond;

 $R^{12}$  is a  $C_{1.6}$  alkyl, substituted with a =O;

55

 $R^{13}$  is  $C_{1-6}$  alkenyl-C(O)OH;  $R^{14}$  is selected from the group consisting of H and  $C_{1-6}$ 

alkyl; and subscript r is an integer from 1 to 10;

with the proviso that when  $R^1$  is 4-bromo-naphthalen-1-yl, 60 and n is 1,  $R^2$  is other than unsubstituted pyrid-2-yl

The present invention also provides an expression cassette comprising a promoter operably linked to a polynucleotide encoding a PYR/PYL receptor polypeptide, wherein introduction of the expression cassette into a plant results in the 65 plant having improved stress tolerance compared to a plant lacking the expression cassette.

In some embodiments, the PYR/PYL receptor polypeptide comprises one or more of SEQ ID NOs:1, 91, 92, 93, 94, 95, 96, 97, 98, 99, 100, 101, 102, 103, 104, 105, 106, 107 and/or 138.

In some embodiments, the PYR/PYL receptor polypeptide is at least 70% (e.g., at least 70%, 80%, 90%, 95%) identical to any of SEQ ID NOs:2-90 or 108-137.

In some embodiments, the PYR/PYL receptor polypeptide is a constitutively-active form such that the receptor will bind a type 2 protein phosphatase (PP2C) in a yeast two-hybrid assay in the absence of abscisic acid or an ABA agonist.

In some embodiments, the PYR/PYL receptor polypeptide bind a type 2 protein phosphatase (PP2C) in a yeast two-hybrid assay in the presence, but not in the absence, of abscisic acid or an ABA agonist.

In some embodiments, the plant has improved drought tolerance compared to a plant lacking the expression cassette.

In some embodiments, the promoter is a root-specific promoter. In some embodiments, the promoter is specific for an aerial portion of the plant. In some embodiments, the promoter is inducible.

The present invention also provides for expression vectors comprising an expression cassette of the invention (e.g., as described above).

The present invention also provides for methods of making a plant with increased stress tolerance. In some embodiments, the method comprises:

introducing the an expression cassette of the invention (e.g., as described above) into a plurality of plants; and

selecting a plant comprising the expression cassette having increased stress tolerance compared to a plant lacking the expression cassette.

The present invention also provides an agricultural chemical formulation formulated for contacting to plants, the formulation comprising a compound selected from the following formulas:

$$\begin{array}{c|c} \mathbf{C} & & & & & & \\ \mathbf{R}^1 - \mathbf{S} & \mathbf{N} - & & & & \\ \mathbf{I} & \mathbf{I} & & & & \\ \mathbf{O} & \mathbf{R}^3 & & & & & \\ \end{array}$$

$$\mathbb{R}^6 - \mathbb{N} \stackrel{\text{O}}{\longrightarrow} \mathbb{C} \mathbb{N}$$

$$(\mathbb{R}^{10})_p \underbrace{\hspace{1cm}}_{m} (\mathbb{R}^{11})_r$$

wherein

R<sup>1</sup> is selected from the group consisting of aryl and heteroaryl, optionally substituted with 1-3 R<sup>1a</sup> groups;

each R<sup>1a</sup> is independently selected from the group consisting of H, halogen,  $C_{1-6}$  alkyl,  $C_{1-6}$  haloalkyl,  $C_{2-6}$  alkenyl,  $C_{2-6}$  alkynyl,  $C_{1-6}$  alkoxy,  $C_{1-6}$  haloalkoxy,  $C_{1-6}$  15 hydroxyalkyl, —NR'R", —SR', —OH, —CN, —NO<sub>2</sub>, —C(O)R', —C(O)OR', —C(O)NR'R", —N(R')C(O) R'', -N(R')C(O)OR'', -N(R')C(O)NR'R'', -OP(O) $(OR')_2$ ,  $-S(O)_2OR'$ ,  $-S(O)_2NR'R''$ , cycloalkyl, heterocycloalkyl, aryl and heteroaryl, wherein the aryl 20 group is optionally substituted with —NO<sub>2</sub> and the heteroaryl group is optionally substituted with  $C_{1-6}$  alkyl;

alternatively, adjacent  $R^{1a}$  groups can combine to form a member selected from the group consisting of cycloalkyl, heterocycloalkyl, aryl and heteroaryl, 25 wherein the aryl group is optionally substituted with –OH;

R' and R" are each independently selected from the group consisting of H and  $C_{1-6}$  alkyl;

 $R^2$  is selected from the group consisting of  $C_{2-6}$  alkenyl, 30 cycloalkenyl, aryl and heteroaryl;

 $R^3$  is H or is optionally combined with  $R^2$  and the atoms to which each is attached to form a heterocycloalkyl optionally substituted with 1-3 R<sup>1a</sup> groups;

 $R^4$  is a heteroaryl, optionally substituted with 1-3  $R^{1a}$  35

 $\ensuremath{\mathrm{R}^5}$  is selected from the group consisting of  $\ensuremath{\mathrm{C}_{1\text{--}6}}$  alkyl and aryl, wherein the aryl is optionally substituted with 1-3 R<sup>1a</sup> groups;

each of R<sup>6</sup> and R<sup>7</sup> are independently selected from the 40 group consisting of aryl and heteroaryl, each optionally substituted with 1-3  $R^{1a}$  groups;

R<sup>8</sup> is selected from the group consisting of cycloalkyl and aryl, each optionally substituted with 1-3 R<sup>1a</sup> groups;

 $R^9$  is H or is optionally combined with a  $R^{1a}$  group of  $R^8$  45 and the atoms to which each is attached to form a heterocycloalkyl; subscript n is 0-2;

X is absent or is selected from the group consisting of –O—, and —N(R')—;

-C(O) and -C(R',R'');

Z is absent or is selected from the group consisting of -N and -C(S) -N(R'), such that one of Y and Z is absent:

each of  $R^{1\dot{0}}$  and  $R^{11}$  are independently selected from the  $\,$  55 group consisting of H, C  $_{1-6}$  alkyl, —C(O)OR', and C  $_{1-6}$  alkenyl-C(O)OH, wherein at least two of the R  $^{10}$  and R  $^{11}$ groups are  $C_{\rm 1-6}$  alkyl and at least one of the  $R^{\rm 10}$  and  $R^{\rm 11}$ groups is C<sub>1-6</sub> alkenyl-C(O)OH;

alternatively, two R<sup>10</sup> or R<sup>11</sup> groups attached to the same 60 carbon are combined to form =O;

alternatively, one R10 group and one R11 group are combined to form a cycloalkyl having from 3 to 6 ring members:

each of subscripts k and m is an integer from 1 to 3, such 65 that the sum of k and m is from 3 to 4;

each of subscripts p and r is an integer from 1 to 10;

wherein two of the R10 and R11 groups on adjacent carbons are combined to form a bond;

 $R^{12}$  is a  $C_{1-6}$  alkyl, substituted with a =O;

 $R^{13}$  is  $C_{1-6}$  alkenyl-C(O)OH;

 $\rm R^{14}$  is selected from the group consisting of H and  $\rm C_{1-6}$ alkvl: and

subscript r is an integer from 1 to 10;

with the proviso that when R<sup>1</sup> is 4-bromo-naphthalen-1-yl, and n is 1, R<sup>2</sup> is other than unsubstituted pyrid-2-yl

In some embodiments, the formulation further comprises at least one of an herbicide, fungicide, pesticide, or fertilizer. In some embodiments, the formulation further comprises a surfactant.

The present invention also provides for a method of increasing stress tolerance in a plant, the method comprising contacting a plant with a sufficient amount of a formulation as described above to increase stress tolerance in the plant compared to not contacting the plant with the compound.

In some embodiments, the contacting step comprises delivering the formulation to the plant by aircraft or irrigation.

The present invention also provides for a method of identifying an agent that agonizes a PYR/PYL polypeptide. In some embodiments, the method comprises

contacting one or more agents to a PYR/PYL polypeptide; and

determining whether the one or more agents bind to and/or or activate the PYR/PYL receptor polypeptide, wherein binding or activation identifies the agent as an agonist of the PYR/PYL polypeptide.

In some embodiments, the determining step comprises contacting the agent to a cell comprising a two-hybrid system, wherein the two-hybrid systems detects interaction of the PYR/PYL polypeptide to a type 2 protein phosphatase (PP2C), wherein agent-dependent interaction of the PYR/ PYL polypeptide to the PP2C identifies the agent as an agonist of the PYR/PYL polypeptide.

# BRIEF DESCRIPTION OF THE DRAWINGS

FIG. 1. Pyrabactin is a seed selective ABA agonist. (A) Structures of molecules described in this study. (B) Pyrabactin activity is suppressed by abi1-1. Seeds of the genotype shown at top were imbibed on media containing 25 µM pyrabactin and scored for germination 4 days after stratification. Shown at bottom are IC<sub>50</sub> values for pyrabactin's germination effect on the genotypes characterized. (C) Microarray comparison of pyrabactin and ABA treatments in seeds. The Y-axis plots the log, transformed value for a probe's response to 25 µM pyrabactin (relative to untreated control) and the Y is absent or is selected from the group consisting of 50 X-axis a probe's response to 1 µM ABA. Plotted are data for probe sets that showed significant responsiveness to ABA or pyrabactin, after removing germination responsive transcripts. (D) Microarray comparison of cycloheximide and ABA responses in seeds. This plot shows the response of the same probe sets analyzed in panel C, but the comparisons are to mRNAs from cycloheximide treated seeds (y-axis). (E) Microarray comparison of pyrabactin and ABA responses in seedlings. Seven-day old seedlings were transferred to 10 µM ABA or 50 µM pyrabactin containing plates for 24 hours and then mRNA samples profiled. Inset in each scatter plot is the Pearson correlation coefficient for each comparison. Detailed microarray methods are described in the Examples section.

FIG. 2. PYR1 encodes an ABA responsive START-domain protein. (A) Pyr1 alleles. Shown are the allele names, strain names (in parentheses) and amino acid changes caused by the Pyr1 mutant alleles identified by screening for pyrabactin resistant mutations. (B) Pictographic representation of Pyr1

and Pyl1-Pyl4 expression values housed in public microarray databases. The heatmap shown at top right is for the first upper three panels and the bottom heatmap for the guard cell data. Plots were made using the eFP browser (D. Winter et al., PLoS ONE 2, e718 (2007)). (C) 35S::GFP-PYR1 comple- 5 ments pyr1-1. Seeds of the genotypes shown were stratified 4 days on 25 µM pyrabactin and then germinated at RT, 90% RH for 3 days in darkness. The Columbia wild type is unable to germinate under these conditions, but pyr1-1 does because it is resistant to pyrabactin. Introduction of a 35S::GFP-PYR1 10 construct into the pyr1-1 genetic background restores pyrabactin sensitivity, which indicates that the GFP fusion protein is functional. (D) Pyr/Pyls are required for normal ABAinduced gene expression in seedlings. Shown are qRT-PCR results for the ABA-responsive gene RD29. L, Ler; C, Col; 15 and Q, quadruple mutant. (E) Pyr/Pyl genes are required for normal ABA-induced stress-induced gene expression in seedlings. Shown are qRT-PCR results for two ABA-responsive taqman probes, as described in the Examples section. L=Ler, C=Col, O=quadruple mutant.

FIG. 3. ABA promotes PYR/PYL binding to PP2Cs. (A) Characterization of the PYR/PYL protein interactions with HAB1. Shown are X-gal stains of yeast colonies grown on plates containing the compounds shown at top. The *Arabidopsis* Genome Initiative (AGI) annotations for each PYR/ 25 PYL gene characterized is shown at the right of the panel. Not tested were PYL8 (AT5G53160) and PYL13 (AT4G18620). Each strain tested expresses an AD-HAB1 fusion protein and the BD-fusion shown at left. Chemicals were tested at 10 μM with the exception of epi-brassinolide (50 nM). (B) PYR1 30 mutant proteins are defective in their interactions with HAB1. 3 PYR1 amino acid substitution mutants that display strong pyrabactin insensitivity in *Arabidopsis* seeds were tested for their interactions with HAB1 in the Y2H. (C) PYR1 interacts with AB11 and AB12 but not the mutant protein encoded by 35 abi2-1

FIG. 4. GFP-PYR1 localizes to the cytoplasm and nucleoplasm. Confocal images are shown of a 35S::GFP-PYR1 construct in the pyr1-1 mutant background. This construct complements the pyrabactin insensitivity phenotype of the 40 pyr1-1 mutant.

FIG. **5**. Pyr1 and Pyl1, 2 and 4 function redundantly in ABA perception. (A) ABA responses in the triple and quadruple mutant lines are altered during germination. Seeds of the genotypes shown at top were stratified 4 days on media 45 containing 0.9  $\mu$ M (+)-ABA and then photographed 3 days after germination in darkness. The short hypocotyl observed in the quadruple mutant when germinated on (+)-ABA is due to the presence of the erecta mutation that is tightly linked to the pyl2-1 insertion allele. (B) ABA responses in the triple 50 and quadruple mutant lines are altered during root growth. Seeds of the genotypes shown at top were stratified 4 days and then transferred to darkness (RT, 90% RH). After 30 hours, seeds with radicle emergence were transferred to plates contain 10  $\mu$ M (+)-ABA and their roots photographed after an 35 additional 3 days growth in the dark.

FIG. **6**. PYR1 is an ABA receptor that regulates PP2C activity. (A) Reconstitution of ABA perception in vitro. Pull-down assays using GST-HAB1 and  $6\times$ His-PYR1 (or mutants) were conducted using purified recombinant proteins 60 (left panel). GST-ABI1 and ABI2 were additionally tested in pull-downs using purified  $6\times$ His-PYR1 (or mutants) and crude lysates containing the PP2Cs shown.  $10~\mu$ M (+)-ABA was used. (B) PYR1 inhibits PP2C activity in the presence of ABA. The PP2C activity of GST-HAB1 was tested in the 65 presence or PYR1 or PYR1<sup>P88S</sup> at different concentrations of ABA using the substrate pNPP. (C) ABA/PYL4-dependent

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inhibition of HAB1 PP2C activity. Recombinant PYL4 (refolded from inclusion bodies) and HAB1 were used in PP2C assays as described. Activity was measured for GST-HAB1 using the phosphatase substrate pNPP. Phosphatase initial reaction velocities were calculated in triplicate by monitoring reactions over time using a plate reader in triplicate and used to calculate activities. The top panel shows the full concentration ranged studied; bottom panel a zoomed region of the lower concentrations tested. The specific activity of the GST-HAB1 used in these experiments was 452.4±12.3  $\mu$ mol/min/mg. Points plotted use ±SD as error bars.

FIG. 7. Proposed model for PYR/PYL control of ABA signaling. Without intending to limit the scope of the invention, we propose the following model: In the absence of ABA (left), PYR/PYL proteins show low binding to PP2Cs, and therefore, PP2C activity is high, which prevents phosphorylation and activation of SnRK2s and downstream factors (DFs). In the presence of ABA, PYR/PYLs bind and inhibit PP2Cs. This allows accumulation of phosphorylated downstream factors and ABA transcriptional responses. The regulation of SnRK2s by PYR/PYLs may be indirect or may involve other factors.

FIG. 8. Activity of small molecule ABA agonists. This figure summarizes data from screening small molecules for receptor activity of PYR1, PYL1, PYL2, PYL3, and PYL4.

FIG. 9. IC50 values for some compounds identified in the PP2C yeast two-hybrid assay. Compound numbers listed in left column correspond to compounds identified in the assay summarized in FIG. 9. Compound 7653159 corresponds to compound 7 in FIG. 9; compound 6655097 corresponds to compound 6 in FIG. 9; and compound 7561035 corresponds to compound 9 in FIG. 9. For each compound, the ability of the compound to agonize PYR/PYL inhibition of the PP2C HAB1 was assessed using a phosphatase assay with the phosphatase substrate pNPP.

FIG. 10. Table of ABA-related phenotypes in the PYL4 overexpression line. PYL4-overexpressing and pyr1;pyl1; pyl2;pyl4 quadruple mutant *Arabidopsis* plants were examined for changes in stress response associated traits including flowering time, stature, chlorophyll content, and wiltiness relative to control *Arabidopsis* plants. Full details for the construction of the mutant plants are provided in the Examples section.

FIG. 11. Alignment of PYR1 and homologs from Arabidopsis. This figure provides an alignment of Arabidopsis PYR/PYL protein sequences. The alignment displays, for example, absolutely conserved amino acids as well as amino acids at positions that are typically conserved. Sequences in the figure include the following PYR/PYL polypeptides: PYL12 (SEQ ID NO:77), PYL8 (SEQ ID NO:78), PYL7 (SEQ ID NO:79), PYL9 (SEQ ID NO:80), PYL11 (SEQ ID NO:81) PYL10 (SEQ ID NO:82), PYL13 (SEQ ID NO:83), PYL5 (SEQ ID NO:84), PYL4 (SEQ ID NO:85), PYL6 (SEQ ID NO:86), PYL2 (SEQ ID NO:87), PYL3 (SEQ ID NO:88), PYR1 (SEQ ID NO:89), and PYL1 (SEQ ID NO:90). Consensus sequences derived from specified members are set forth below the alignment. ALL\_Con=SEQ ID NOS:93-95; 1\_12\_Con=SEQ ID NOS:96-99; 1\_6\_Con=SEQ ID NOS: 100, 139 and 102; 7\_10\_Con=SEQ ID NOS:103 and 140; 11\_13\_Con=SEQ ID NOS:106 and 107.

FIG. 12. Activity of additional ABA agonists. The listed compounds include the naturally-occurring plant compound artemisinic acid, as well as analogs thereof.

# **DEFINITIONS**

The term "promoter," as used herein, refers to a polynucleotide sequence capable of driving transcription of a coding

sequence in a cell. Thus, promoters used in the polynucleotide constructs of the invention include cis-acting transcriptional control elements and regulatory sequences that are involved in regulating or modulating the timing and/or rate of transcription of a gene. For example, a promoter can be a cis- 5 acting transcriptional control element, including an enhancer, a promoter, a transcription terminator, an origin of replication, a chromosomal integration sequence, 5' and 3' untranslated regions, or an intronic sequence, which are involved in transcriptional regulation. These cis-acting sequences typically interact with proteins or other biomolecules to carry out (turn on/off, regulate, modulate, etc.) gene transcription. A "plant promoter" is a promoter capable of initiating transcription in plant cells. A "constitutive promoter" is one that is capable of initiating transcription in nearly all tissue types, 15 whereas a "tissue-specific promoter" initiates transcription only in one or a few particular tissue types.

The term "plant" includes whole plants, shoot vegetative organs and/or structures (e.g., leaves, stems and tubers), roots, flowers and floral organs (e.g., bracts, sepals, petals, 20 stamens, carpels, anthers), ovules (including egg and central cells), seed (including zygote, embryo, endosperm, and seed coat), fruit (e.g., the mature ovary), seedlings, plant tissue (e.g., vascular tissue, ground tissue, and the like), cells (e.g., guard cells, egg cells, trichomes and the like), and progeny of 25 same. The class of plants that can be used in the method of the invention is generally as broad as the class of higher and lower plants amenable to transformation techniques, including angiosperms (monocotyledonous and dicotyledonous plants), gymnosperms, ferns, and multicellular algae. It 30 includes plants of a variety of ploidy levels, including aneuploid, polyploid, diploid, haploid, and hemizygous.

A polynucleotide sequence is "heterologous" to an organism or a second polynucleotide sequence if it originates from a foreign species, or, if from the same species, is modified 35 from its original form. For example, when a promoter is said to be operably linked to a heterologous coding sequence, it means that the coding sequence is derived from one species whereas the promoter sequence is derived another, different species; or, if both are derived from the same species, the 40 coding sequence is not naturally associated with the promoter (e.g., is a genetically engineered coding sequence, e.g., from a different gene in the same species, or an allele from a different ecotype or variety).

A polynucleotide "exogenous" to an individual plant is a 45 polynucleotide which is introduced into the plant by any means other than by a sexual cross. Examples of means by which this can be accomplished are described below, and include *Agrobacterium*-mediated transformation, biolistic methods, electroporation, and the like. Such a plant containing the exogenous nucleic acid is referred to here as a  $T_1$  (e.g., in *Arabidopsis* by vacuum infiltration) or Ro (for plants regenerated from transformed cells in vitro) generation transgenic plant.

As used herein, the term "transgenic" describes a nonnaturally occurring plant that contains a genome modified by
man, wherein the plant includes in its genome an exogenous
nucleic acid molecule, which can be derived from the same or
a different plant species. The exogenous nucleic acid molecule can be a gene regulatory element such as a promoter, 60
enhancer, or other regulatory element, or can contain a coding
sequence, which can be linked to a heterologous gene regulatory element. Transgenic plants that arise from sexual cross
or by selfing are descendants of such a plant and are also
considered "transgenic.".

An "expression cassette" refers to a nucleic acid construct that, when introduced into a host cell, results in transcription 10

and/or translation of an RNA or polypeptide, respectively. Antisense or sense constructs that are not or cannot be translated are expressly included by this definition. In the case of both expression of transgenes and suppression of endogenous genes (e.g., by antisense, or sense suppression) one of skill will recognize that the inserted polynucleotide sequence need not be identical, but may be only "substantially identical" to a sequence of the gene from which it was derived. As explained below, these substantially identical variants are specifically covered by reference to a specific nucleic acid sequence.

"Increased" or "enhanced" PYR/PYL expression or activity refers to an augmented change in the protein's expression or activity. Examples of such increased activity or expression include, e.g., where PYR/PYL expression is increased above control levels and/or where it is ectopically expressed, e.g., in a place or time where it is not expressed in a control. In some embodiments, PYR/PYL expression or activity is increased above the level of that in wild-type, non-transgenic control plants (i.e., the quantity of PYR/PYL activity or expression of the PYR/PYL gene is increased). In some embodiments, PYR/PYL expression or activity can be present, for example, in an organ, tissue, or cell where it is not normally detected in wild-type, non-transgenic control plants (i.e., PYR/PYL expression or activity is increased within certain tissue types). In some embodiments, PYR/PYL expression or activity is increased when its expression or activity is present in an organ, tissue or cell for a longer period than in a wild-type, non-transgenic controls (i.e., duration of PYR/PYL expression or activity is increased).

Two nucleic acid sequences or polypeptides are said to be "identical" if the sequence of nucleotides or amino acid residues, respectively, in the two sequences is the same when aligned for maximum correspondence as described below. The terms "identical" or percent "identity," in the context of two or more nucleic acids or polypeptide sequences, refer to two or more sequences or subsequences that are the same or have a specified percentage of amino acid residues or nucleotides that are the same, when compared and aligned for maximum correspondence over a comparison window, as measured using one of the following sequence comparison algorithms or by manual alignment and visual inspection. When percentage of sequence identity is used in reference to proteins or peptides, it is recognized that residue positions that are not identical often differ by conservative amino acid substitutions, where amino acids residues are substituted for other amino acid residues with similar chemical properties (e.g., charge or hydrophobicity) and therefore do not change the functional properties of the molecule. Where sequences differ in conservative substitutions, the percent sequence identity may be adjusted upwards to correct for the conservative nature of the substitution. Means for making this adjustment are well known to those of skill in the art. Typically this involves scoring a conservative substitution as a partial rather than a full mismatch, thereby increasing the percentage sequence identity. Thus, for example, where an identical amino acid is given a score of 1 and a non-conservative substitution is given a score of zero, a conservative substitution is given a score between zero and 1. The scoring of conservative substitutions is calculated according to, e.g., the algorithm of Meyers & Miller, Computer Applic. Biol. Sci. 4:11-17 (1988) e.g., as implemented in the program PC/GENE (Intelligenetics, Mountain View, Calif., USA).

The phrase "substantially identical," used in the context of two nucleic acids or polypeptides, refers to a sequence that has at least 25% sequence identity with a reference sequence. Alternatively, percent identity can be any integer from 25% to

100%. Some embodiments include at least: 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, or 99%, compared to a reference sequence using the programs described herein; preferably BLAST using standard parameters, as described below. The present invention provides for nucleic acids encoding polypeptides that are substantially identical to any of SEQ ID NO:2-90 or 108-137.

For sequence comparison, typically one sequence acts as a reference sequence, to which test sequences are compared. When using a sequence comparison algorithm, test and reference sequences are entered into a computer, subsequence coordinates are designated, if necessary, and sequence algorithm program parameters are designated. Default program parameters can be used, or alternative parameters can be designated. The sequence comparison algorithm then calculates the percent sequence identities for the test sequences relative to the reference sequence, based on the program parameters.

A "comparison window", as used herein, includes reference to a segment of any one of the number of contiguous 20 positions selected from the group consisting of from 20 to 600, usually about 50 to about 200, more usually about 100 to about 150 in which a sequence may be compared to a reference sequence of the same number of contiguous positions after the two sequences are optimally aligned. Methods of 25 alignment of sequences for comparison are well-known in the art. Optimal alignment of sequences for comparison can be conducted, e.g., by the local homology algorithm of Smith & Waterman, Adv. Appl. Math. 2:482 (1981), by the homology alignment algorithm of Needleman & Wunsch, J. Mol. Biol. 48:443 (1970), by the search for similarity method of Pearson & Lipman, Proc. Nat'l. Acad. Sci. USA 85:2444 (1988), by computerized implementations of these algorithms (GAP, BESTFIT, FASTA, and TFASTA in the Wisconsin Genetics Software Package, Genetics Computer Group, 575 Science 35 Dr., Madison, Wis.), or by manual alignment and visual inspection.

Examples of algorithms that are suitable for determining percent sequence identity and sequence similarity are the BLAST and BLAST 2.0 algorithms, which are described in 40 Altschul et al. (1990) J. Mol. Biol. 215: 403-410 and Altschul et al. (1977) Nucleic Acids Res. 25: 3389-3402, respectively. Software for performing BLAST analyses is publicly available through the National Center for Biotechnology Information (NCBI) web site. The algorithm involves first identifying 45 high scoring sequence pairs (HSPs) by identifying short words of length W in the query sequence, which either match or satisfy some positive-valued threshold score T when aligned with a word of the same length in a database sequence. T is referred to as the neighborhood word score 50 threshold (Altschul et al, supra). These initial neighborhood word hits acts as seeds for initiating searches to find longer HSPs containing them. The word hits are then extended in both directions along each sequence for as far as the cumulative alignment score can be increased. Cumulative scores 55 are calculated using, for nucleotide sequences, the parameters M (reward score for a pair of matching residues; always >0) and N (penalty score for mismatching residues; always <0). For amino acid sequences, a scoring matrix is used to calculate the cumulative score. Extension of the word hits in each 60 direction are halted when: the cumulative alignment score falls off by the quantity X from its maximum achieved value; the cumulative score goes to zero or below, due to the accumulation of one or more negative-scoring residue alignments; or the end of either sequence is reached. The BLAST algo- 65 rithm parameters W, T, and X determine the sensitivity and speed of the alignment. The BLASTN program (for nucle12

otide sequences) uses as defaults a word size (W) of 28, an expectation (E) of 10, M=1, N=-2, and a comparison of both strands. For amino acid sequences, the BLASTP program uses as defaults a word size (W) of 3, an expectation (E) of 10, and the BLOSUM62 scoring matrix (see Henikoff & Henikoff, Proc. Natl. Acad. Sci. USA 89:10915 (1989)).

The BLAST algorithm also performs a statistical analysis of the similarity between two sequences (see, e.g., Karlin & Altschul, Proc. Nat'l. Acad. Sci. USA 90:5873-5787 (1993)). One measure of similarity provided by the BLAST algorithm is the smallest sum probability (P(N)), which provides an indication of the probability by which a match between two nucleotide or amino acid sequences would occur by chance. For example, a nucleic acid is considered similar to a reference sequence if the smallest sum probability in a comparison of the test nucleic acid to the reference nucleic acid is less than about 0.01, more preferably less than about  $10^{-5}$ , and most preferably less than about  $10^{-20}$ .

"Conservatively modified variants" applies to both amino acid and nucleic acid sequences. With respect to particular nucleic acid sequences, conservatively modified variants refers to those nucleic acids which encode identical or essentially identical amino acid sequences, or where the nucleic acid does not encode an amino acid sequence, to essentially identical sequences. Because of the degeneracy of the genetic code, a large number of functionally identical nucleic acids encode any given protein. For instance, the codons GCA, GCC, GCG and GCU all encode the amino acid alanine. Thus, at every position where an alanine is specified by a codon, the codon can be altered to any of the corresponding codons described without altering the encoded polypeptide. Such nucleic acid variations are "silent variations," which are one species of conservatively modified variations. Every nucleic acid sequence herein which encodes a polypeptide also describes every possible silent variation of the nucleic acid. One of skill will recognize that each codon in a nucleic acid (except AUG, which is ordinarily the only codon for methionine) can be modified to yield a functionally identical molecule. Accordingly, each silent variation of a nucleic acid which encodes a polypeptide is implicit in each described sequence.

As to amino acid sequences, one of skill will recognize that individual substitutions, in a nucleic acid, peptide, polypeptide, or protein sequence which alters a single amino acid or a small percentage of amino acids in the encoded sequence is a "conservatively modified variant" where the alteration results in the substitution of an amino acid with a chemically similar amino acid. Conservative substitution tables providing functionally similar amino acids are well known in the art.

The following six groups each contain amino acids that are conservative substitutions for one another:

- 1) Alanine (A), Serine (S), Threonine (T);
- 2) Aspartic acid (D), Glutamic acid (E);
- 3) Asparagine (N), Glutamine (Q);
- 4) Arginine (R), Lysine (K);
- 5) Isoleucine (I), Leucine (L), Methionine (M), Valine (V);
- 6) Phenylalanine (F), Tyrosine (Y), Tryptophan (W). (see, e.g., Creighton, Proteins (1984)).

As used herein, the term "drought-resistance" or "droughttolerance," including any of their variations, refers to the ability of a plant to recover from periods of drought stress (i.e., little or no water for a period of days). Typically, the drought stress will be at least 5 days and can be as long as, for example, 18 to 20 days or more (e.g., at least 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20 days), depending on, for example, the plant species.

## I. Introduction

The present invention is based, in part, on the discovery of selective abscisic acid (ABA) agonist small organic molecules as well as a protein, PYR1, which is required for the ABA agonist activity. It has further been discovered that PYR1 is a member of the PYR/PYL receptor protein family. Plants examined to date express more than one PYR/PYL receptor protein family members and have at least somewhat redundant activity. Increasing expression or activity of one or more PYR/PYL protein in a plant therefore will result in increased ABA sensitivity and accordingly improved stress (e.g. cold, heat, salinity, or drought) response and tolerance as well as other desirable ABA-mediated phenotypes.

Abscisic acid is a multifunctional phytohormone involved in a variety of phyto-protective functions including bud dormancy, seed dormancy and/or maturation, abscission of leaves and fruits, and response to a wide variety of biological stresses (e.g. cold, heat, salinity, and drought). ABA is also responsible for regulating stomatal closure by a mechanism independent of CO<sub>2</sub> concentration. Thus, because PYR/PYL ABA receptor proteins mediate ABA signalling, these phenotypes can be modulated by modulating expression of PYR/ PYL. Phenotypes that are induced by ABA can be increased or speeded in plants with increased expression of PYR/PYL whereas such phenotypes can be reduced or slowed in plants with decreased expression of PYR/PYL. PYR/PYL mediates ABA signaling as a positive regulator in, for example, seed germination, post-germination growth, stomatal movement and plant tolerance to stress including, but not limited to, drought. Accordingly, when abscisic acid sensitivity is increased by overexpressing PYR/PYL, desirable characteristics in plants such as increased stress (e.g., drought) tolerance and delayed seed germination is achieved. Other desirable characteristics that can be generated in the plants of the invention include, e.g., a change in flowering time and/or increased chlorophyll content.

# II. ABA Agonists

The present invention provides for small molecule ABA agonists, i.e., compounds that activate PYR/PYL proteins. Exemplary ABA agonists include, e.g., a compound selected from the following formulas:

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$$R^4$$
— $S$ — $CH_2$ — $X$ — $R^5$ 

$$R^6 - N$$
CN
(III) 55

$$R^7-Y$$
 $N-Z-R^8$ 
 $R^9$ 
 $R^9$ 

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-continued

$$(\mathbb{R}^{10})_p \longrightarrow (\mathbb{R}^{11})_r \tag{V}$$

$$\mathbb{R}^{12} \xrightarrow{(\mathbb{R}^{14})_r} \mathbb{R}^{13}$$

wherein

R<sup>1</sup> is selected from the group consisting of aryl and heteroaryl, optionally substituted with 1-3 R<sup>1a</sup> groups;

each  $R^{1a}$  is independently selected from the group consisting of H, halogen,  $C_{1-6}$  alkyl,  $C_{1-6}$  haloalkyl,  $C_{2-6}$  alkenyl,  $C_{2-6}$  alkynyl,  $C_{1-6}$  alkoxy,  $C_{1-6}$  haloalkoxy,  $C_{1-6}$  hydroxyalkyl, —NR'R", —SR', —OH, —CN, —NO<sub>2</sub>, —C(O)R', —C(O)OR', —C(O)NR'R", —N(R')C(O) R", —N(R')C(O)OR", —N(R')C(O)NR'R", —OP(O) (OR')<sub>2</sub>, —S(O)<sub>2</sub>OR', —S(O)<sub>2</sub>NR'R", cycloalkyl, heterocycloalkyl, aryl and heteroaryl, wherein the aryl group is optionally substituted with —NO<sub>2</sub> and the heteroaryl group is optionally substituted with C<sub>1-6</sub> alkyl;

alternatively, adjacent R<sup>1a</sup> groups can combine to form a member selected from the group consisting of cycloalkyl, heterocycloalkyl, aryl and heteroaryl, wherein the aryl group is optionally substituted with —OH:

R' and R" are each independently selected from the group consisting of H and  $C_{1-6}$  alkyl;

R<sup>2</sup> is selected from the group consisting of C<sub>2-6</sub> alkenyl, cycloalkenyl, aryl and heteroaryl;

R<sup>3</sup> is H or is optionally combined with R<sup>2</sup> and the atoms to which each is attached to form a heterocycloalkyl optionally substituted with 1-3 R<sup>1a</sup> groups;

R<sup>4</sup> is a heteroaryl, optionally substituted with 1-3 R<sup>1a</sup> groups;

 $R^{5}$  is selected from the group consisting of  $C_{1-6}$  alkyl and aryl, wherein the aryl is optionally substituted with 1-3  $R^{1a}$  groups;

each of  $R^6$  and  $R^7$  are independently selected from the group consisting of aryl and heteroaryl, each optionally substituted with 1-3  $R^{1a}$  groups;

R<sup>8</sup> is selected from the group consisting of cycloalkyl and aryl, each optionally substituted with 1-3 R<sup>1a</sup> groups;

R<sup>9</sup> is H or is optionally combined with a R<sup>1a</sup> group of R<sup>8</sup> and the atoms to which each is attached to form a heterocycloalkyl; subscript n is 0-2;

X is absent or is selected from the group consisting of —O—, and —N(R')—;

Y is absent or is selected from the group consisting of —C(O)— and —C(R',R")—;

Z is absent or is selected from the group consisting of —N=, and —C(S)—N(R')—, such that one of Y and Z is absent;

each of  $R^{10}$  and  $R^{11}$  are independently selected from the group consisting of H,  $C_{1\text{-}6}$  alkyl, —C(O)OR', and  $C_{1\text{-}6}$  alkenyl-C(O)OH, wherein at least two of the  $R^{10}$  and  $R^{11}$  groups are  $C_{1\text{-}6}$  alkyl and at least one of the  $R^{10}$  and  $R^{11}$  groups is  $C_{1\text{-}6}$  alkenyl-C(O)OH;

alternatively, two R<sup>10</sup> or R<sup>11</sup> groups attached to the same carbon are combined to form ==O;

alternatively, one R10 group and one R11 group are combined to form a cycloalkyl having from 3 to 6 ring

each of subscripts k and m is an integer from 1 to 3, such that the sum of k and m is from 3 to 4;

each of subscripts p and r is an integer from 1 to 10; wherein two of the R10 and R11 groups on adjacent carbons are combined to form a bond;

 $R^{12}$  is a  $C_{1-6}$  alkyl, substituted with a  $\Longrightarrow$ O;

 $R^{13}$  is  $C_{1-6}$  alkenyl-C(O)OH;

 ${\rm R}^{14}$  is selected from the group consisting of H and  ${\rm C}_{1\text{-}6}$ 

subscript r is an integer from 1 to 10;

with the proviso that when R<sup>1</sup> is 4-bromo-naphthalen-1-yl, and n is 1, R<sup>2</sup> is other than unsubstituted pyrid-2-yl

Exemplary compounds are further depicted in the Examples and Figures. See, e.g., FIGS. 9, 10, and 13.

The ABA agonist compounds of the present invention can be prepared by a variety of methods known to one of skill in the art. For example, the sulphonamide compounds can be 20 prepared by reaction of a sulfonyl chloride and an amine to provide the sulphonamide. Amide compounds of the present invention can be prepared in a similar fashion using an acid chloride in place of the sulfonyl chloride, or carbodiimide coupling reagents known to one of skill in the art. Additional 25 methods of making the compounds of the present invention are known to one of skill in the art, for example, those described in Comprehensive Organic Transformations, 2d ed., Richard C. Larock, 1999. The starting materials for the methods described above are commercially available (Sigma-30 Aldrich) or can be prepared by methods known to one of skill

Phenotypes that are induced by ABA can be increased or speeded in plants (or plant parts such as seeds) by contacting the plants with a sufficient amount of an ABA agonist of the 35 invention to induce the ABA-inducible phenotypes. ABA agonists of the invention are useful as, e.g., positive enhancers of, for example, delayed seed germination, post-germination growth, stomatal movement and plant tolerance to stress including, but not limited to, drought.

III. ABA Agonist Formulations

The present invention provides for agricultural chemical formulation formulated for contacting to plants, wherein the formulation comprises an ABA agonist of the present invention. In some embodiments, the plants that are contacted with 45 the agonists do not comprise or express a heterologous PYR/ PYL polypeptide (e.g., the plants are not transgenic or are transgenic but express heterologous proteins other than heterologous PYR/PYL proteins). In some embodiments, the plants that are contacted with the agonists do comprise or 50 express a heterologous PYR/PYL polypeptide as described herein.

The formulations can be suitable for treating plants or plant propagation material, such as seeds, in accordance with the present invention, e.g., in a carrier. Suitable additives include 55 buffering agents, wetting agents, coating agents, polysaccharides, and abrading agents. Exemplary carriers include water, aqueous solutions, slurries, solids and dry powders (e.g., peat, wheat, bran, vermiculite, clay, pasteurized soil, many forms of calcium carbonate, dolomite, various grades of gypsum, 60 naturally expressed, cloned or synthesized. bentonite and other clay minerals, rock phosphates and other phosphorous compounds, titanium dioxide, humus, talc, alginate and activated charcoal. Any agriculturally suitable carrier known to one skilled in the art would be acceptable and is contemplated for use in the present invention. Optionally, the 65 formulations can also include at least one surfactant, herbicide, fungicide, pesticide, or fertilizer.

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Treatment can be performed using a variety of known methods, e.g., by spraying, atomizing, dusting or scattering the compositions over the propagation material or brushing or pouring or otherwise contacting the compositions over the plant or, in the event of seed, by coating, encapsulating, or otherwise treating the seed. In an alternative to directly treating a plant or seed before planting, the formulations of the invention can also be introduced into the soil or other media into which the seed is to be planted. In some embodiments, a carrier is also used in this embodiment. The carrier can be solid or liquid, as noted above. In some embodiments peat is suspended in water as a carrier of the ABA agonist, and this mixture is sprayed into the soil or planting media and/or over the seed as it is planted.

IV. Screening for New ABA Agonists and Antagonists

The present invention also provides methods of screening for ABA agonists and antagonists by screening for a molecule's ability to induce PYR/PYL-PP2C binding in the case of agonists, or to disrupt the ability of ABA and other agonists to promote PYR/PYL-PP2C binding in the case of antagonists. A number of different screening protocols can be utilized to identify agents that agonize or antagonize a PYR/ PYL polypeptide.

Screening can take place using isolated, purified or partially purified reagents. In some embodiments, purified or partially purified PYR/PYL polypeptide can be used.

Alternatively, cell-based methods of screening can be used. For example, cells that naturally-express a PYR/PYL polypeptide or that recombinantly express a PYR/PYL polypeptide can be used. In some embodiments, the cells used are plant cells, animal cells, bacterial cells, fungal cells, including but not limited to yeast cells, insect cells, or mammalian cells. In general terms, the screening methods involve screening a plurality of agents to identify an agent that modulates the activity of a PYR/PYL polypeptide by, e.g., binding to PYR/PYL polypeptide, or activating a PYR/PYL polypeptide or increasing expression of a PYR/PYL polypeptide, or a transcript encoding a PYR/PYL polypeptide.

1. PYR/PYL Polypeptide Binding Assays

Optionally, preliminary screens can be conducted by screening for agents capable of binding to a PYR/PRL polypeptide, as at least some of the agents so identified are likely PYR/PYL polypeptide modulators.

Binding assays can involve contacting a PYR/PYL polypeptide with one or more test agents and allowing sufficient time for the protein and test agents to form a binding complex. Any binding complexes formed can be detected using any of a number of established analytical techniques. Protein binding assays include, but are not limited to, methods that measure co-precipitation or co-migration on nondenaturing SDS-polyacrylamide gels, and co-migration on Western blots (see, e.g., Bennet, J. P. and Yamamura, H. I. (1985) "Neurotransmitter, Hormone or Drug Receptor Binding Methods," in Neurotransmitter Receptor Binding (Yamamura, H. I., et al., eds.), pp. 61-89. Other binding assays involve the use of mass spectrometry or NMR techniques to identify molecules bound to PYR/PYL polypeptide or displacement of labeled substrates (e.g., labeled ABA). The PYR/PYL polypeptide protein utilized in such assays can be

2. Activity

PYR/PYL polypeptide agonists can be identified by screening for agents that activate or increase activity of a PYR/PYL polypeptide. Antagonists can be identified by reducing activity.

One activity assay involves testing whether a candidate agonist can induce binding of a PYR/PYL protein to a type 2

protein phosphatase (PP2C) polypeptide in an agonist-specific fashion. Mammalian or yeast two-hybrid approaches (see, e.g., Bartel, P. L. et. al. *Methods Enzymol*, 254:241 (1995)) can be used to identify polypeptides or other molecules that interact or bind when expressed together in a cell. In some embodiments, agents that agonize a PYR/PYL polypeptide are identified in a two-hybrid assay between a PYR/PYL polypeptide and a type 2 protein phosphatase (PP2C) polypeptide, wherein an ABA agonist is identified as an agent that activates or enables binding of the PYR/PYL polypeptide and the PP2C polypeptide. Thus, the two polypeptides bind in the presence, but not in the absence of the agent.

In some embodiments, agents that antagonize a PYR/PYL polypeptide are identified in a two-hybrid assay between a PYR/PYL polypeptide and a type 2 protein phosphatase (PP2C) polypeptide, wherein an ABA antagonist is identified as an agent that decreases binding of the PYR/PYL polypeptide and the PP2C polypeptide, optionally in the presence of ABA or a PYR/PYL ABA agonist. Thus, the antagonist blocks the normal binding of the two polypeptides that is normally promoted by ABA or other agonists, or alternatively, that is observed in constitutively interacting PYR/PYL proteins.

# 3. Expression Assays

Screening for a compound that increases the expression of a PYR/PYL polypeptide is also provided. Screening methods generally involve conducting cell-based or plant-based assays in which test compounds are contacted with one or more cells expressing PYR/PYL polypeptide, and then detecting an increase in PYR/PYL expression (either transcript or translation product). Assays can be performed with cells that naturally express PYR/PYL or in cells recombinantly altered to express PYR/PYL, or in cells recombinantly altered to express a reporter gene under the control of the PYR/PYL promoter.

Various controls can be conducted to ensure that an observed activity is authentic including running parallel reactions with cells that lack the reporter construct or by not contacting a cell harboring the reporter construct with test compound.

# 4. Validation

Agents that are initially identified by any of the foregoing 45 screening methods can be further tested to validate the apparent activity and/or determine other biological effects of the agent. In some cases, the identified agent is tested for the ability to effect plant stress (e.g., drought tolerance), seed germination, or another phenotype affected by ABA. A number of such assays and phenotypes are known in the art and can be employed according to the methods of the invention.

# 5. Solid Phase and Soluble High Throughput Assays

In the high throughput assays of the invention, it is possible to screen up to several thousand different modulators or 55 ligands in a single day. In particular, each well of a microtiter plate can be used to run a separate assay against a selected potential modulator, or, if concentration or incubation time effects are to be observed, every 5-10 wells can test a single modulator. Thus, a single standard microtiter plate can assay 60 about 100 (e.g., 96) modulators. If 1536 well plates are used, then a single plate can easily assay from about 100 to about 1500 different compounds. It is possible to assay several different plates per day; assay screens for up to about 6,000-20,000 or more different compounds are possible using the 65 integrated systems of the invention. In addition, microfluidic approaches to reagent manipulation can be used.

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The molecule of interest (e.g., PYR/PYL or a cell expressing a PYR/PYL polypeptide) can be bound to the solid state component, directly or indirectly, via covalent or non covalent linkage.

The invention provides in vitro assays for identifying, in a high throughput format, compounds that can modulate the expression or activity of PYR/PYL.

# V. PYR/PYL Receptor Polypeptides

Polypeptides of the invention, when expressed in plants, mediate ABA and ABA analog signaling. In some embodiments, the PYR/PYL polypeptides interact (e.g., in a yeast two-hybrid assay) with a PP2C polypeptide (e.g., ABI1 or 2 or orthologs thereof, e.g., from the group A subfamily of PP2Cs) in an ABA, pyrabactin, or other ABA agonist—dependent manner as described herein.

A wide variety of PYR/PYL polypeptide sequences are known in the art and can be used according to the methods and compositions of the invention. As noted herein, while PYR1 was originally identified as an ABA receptor in *Arabidopsis*, in fact PYR1 is a member of a group of at least 14 proteins (PYR/PYL proteins) in the same protein family in *Arabidopsis* and that also mediate ABA signaling. This protein family is also present in other plants (see, e.g., SEQUENCE LISTING) is characterized in part by the presence of one or more or all of a polyketide cyclase domain 2 (PF10604), a polyketide cyclase domain 1 (PF03364), and a Bet V I domain (PF03364). START/Bet v 1 superfamily domain are described in, for example, Radauer, *BMC Evol. Biol.* 8:286 (2008).

In situations where variants or orthologs of the above sequences are desired, it can be useful to generate sequence alignments to identify conserved amino acid or motifs (i.e., where alteration in sequences may alter protein function) and regions where variation occurs in alignment of sequences (i.e., where variation of sequence is not likely to significantly affect protein activity). SEQ ID NO:1, 91, and 92 provide consensus sequences useful for identifying PYR/PYL polypeptides. Other useful consensus sequences include, e.g.,

(SEQ ID NO: 138)

EXLXXXDXXXXXXXXXGGXHXL;

(SEQ ID NO: 93)

CXSXXXXXXAPXXXXWXXXXFXXXFXXXC,

(SEQ ID NO: 94)

GXXRXVXXXXXXXPAXXXXEXLXXXD,
and/or

(SEQ ID NO: 95)

GGXHRLXNYXS.

In addition, more specific consensus sequences can be represented by aligning subsets of the 14 members of the *Arabidopsis* PYR/PYL proteins. Examples of such consensus sequences include, e.g.,

PYR1 to PYL12	(SEO	חד	NO ·	961
CxSxxxxxxAPxxxxWxxxxxFxxPxxxKxFxxxC	(DEQ	ıD	110.	50,
GxxRxVxxxSxLPAxxSxExLxxxD	(SEQ	ID	NO :	97)
	(SEO	ID	NO:	98)
GGxHRLxNYxS	~			/
(ESxxVDxPxGNxxxxTxxFxxxxxxxNLxxL	(SEQ	ID	NO :	99)

## -continued

PYR1-PYL6

(SEQ ID NO: 100)

(SEQ ID NO: 101)

VGRxVxVxSGLPAxxSxExLxxxDxxxxxxxFxxxGGxHRLxNYxSVT

(SEQ ID NO: 102)

VxESYxVDxPxGNxxxxTxxFxDxxxxxNLQxL

PYL7-PYL10

(SEQ ID NO: 103) HxHxxxxxQCxSxLVKxIxAPxHxVWSxVRRFDxPQKYKPFxSRCxVxGx

(SEQ ID NO: 104)

ExGxxREVxxKSGLPATxSTExLExLDDxEHILxIXIxGGDHRLKNYSSX

XXXHXEXIXGXXGTX

(SEQ ID NO: 105)

xxESFVVDVPxGNTKxxTCxFVExLIxCNLxSLAxxxERL

PYL11-PYL13

(SEQ ID NO: 106)

CxSxxVxTIxAPLxLVWSILRxFDxPxxxxxFVKxCxxxSGxGG

(SEQ ID NO: 107)

GSVRxVTxVSxxPAxFSxERLxELDDESHVMxxSIIGGxHRLVNYxSKT

Accordingly, in some embodiments, the PYR/PYL polypeptides of the invention comprise one or more of the above-described consensus sequences or conservative variants thereof.

Those of skill in the art will recognize that the variable 30 positions within the above consensus sequences can be selected based on what amino acids occur at their corresponding positions in specific PYR1 polypeptides (e.g., as occur in any of SEQ ID NOs:2-90) or alternatively can be conservative substitutions thereof. In some embodiments, the PYR/PYL polypeptides of the invention are substantially identical to (e.g., at least 70%, 75%, 80%, 85%, 90%, 95% identical to) any of SEQ ID NO:2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 51, 52, 53, 54, 55, 56, 57, 58, 59, 60, 61, 62, 63, 64, 65, 66, 67, 68, 69, 70, 71, 72, 73, 74, 75, 76, 77, 78, 79, 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 108, 109, 110, 111, 112, 113, 114, 115, 116, 117, 118, 119, 120, 121, 122, 123, 124, 125, 126, 45 127, 128, 129, 130, 131, 132, 133, 134, 135, 136, or 137.

The present invention provides for use of the above proteins and/or nucleic acid sequences, encoding such polypeptides, in the methods and compositions (e.g., expression cassettes, plants, etc.) of the present invention. The isolation of a 50 polynucleotide sequence encoding a plant PYR/PYL (e.g., from plants where PYR/PYL sequences have not yet been identified) may be accomplished by a number of techniques. For instance, oligonucleotide probes based on the PYR/PYL coding sequences disclosed (e.g., as listed in the SEQUENCE 55 LISTING) here can be used to identify the desired PYR/PYL gene in a cDNA or genomic DNA library. To construct genomic libraries, large segments of genomic DNA are generated by random fragmentation, e.g., using restriction endonucleases, and are ligated with vector DNA to form concate- 60 mers that can be packaged into the appropriate vector. To prepare a cDNA library, mRNA is isolated from the desired tissue, such as a leaf from a particular plant species, and a cDNA library containing the gene transcript of interest is prepared from the mRNA. Alternatively, cDNA may be prepared from mRNA extracted from other tissues in which PYR/PYL gene is expressed.

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The cDNA or genomic library can then be screened using a probe based upon the sequence of a PYR/PYL gene disclosed here. Probes may be used to hybridize with genomic DNA or cDNA sequences to isolate homologous genes in the same or different plant species. Alternatively, antibodies raised against a polypeptide can be used to screen an mRNA expression library.

Alternatively, the nucleic acids encoding PYR/PYL can be amplified from nucleic acid samples using amplification techniques. For instance, polymerase chain reaction (PCR) technology can be used to amplify the coding sequences of PYR/ PYL directly from genomic DNA, from cDNA, from genomic libraries or cDNA libraries. PCR and other in vitro amplification methods may also be useful, for example, to clone polynucleotide sequences encoding PYR/PYL to be expressed, to make nucleic acids to use as probes for detecting the presence of the desired mRNA in samples, for nucleic acid sequencing, or for other purposes. For a general overview of PCR see PCR Protocols: A Guide to Methods and Applica-20 tions. (Innis, M, Gelfand, D., Sninsky, J. and White, T., eds.), Academic Press, San Diego (1990). Appropriate primers and probes for identifying sequences from plant tissues are generated from comparisons of the sequences provided here with other related genes.

In some embodiments, the partial or entire genome of a number of plants has been sequenced and open reading frames identified. By a BLAST search, one can identify the coding sequence for PYR/PYL in various plants.

Variants from naturally-occurring PYR/PYL polypeptides (or nucleic acids encoding such polypeptides) are contemplated by the term PYR/PYL polypeptide. Variants include, e.g., fusion proteins, deletions or mutations that retain activity.

In some embodiments, the PYR/PYL polypeptide is activated (e.g., as measured in a two-hybrid assay with PP2C or other receptor assays) in the presence of ABA (or ABA agonist) but is not significantly active in the absence of ABA or agonist. Alternatively, in some embodiments, the PYR/PYL polypeptides of the invention are constitutively active, i.e., are active in the absence of ABA or an ABA agonist. As described in the Examples, the inventors have found that the mutations H60P, M158T, M158I, M158S, or M158V in Arabidopsis PYR1 changes the protein to a constitutively active protein. As both of these positions (H60 and M158) are present on the dimer interface of the PYR/PYL protein, it is believed that other constitutive mutants can be generated by introducing amino acid changes at other dimer interface positions (e.g., F61, K63, I84, S85, L87, P88, A89, S152, D155, T156, F159, T162, L166, and/or K170). While the positions above are made with reference to the Arabidopsis PYR1 protein, it is intended that the corresponding position in other PYR/PYL polypeptides are also included in the above description. The corresponding position in another PYR/PYL polypeptide can be readily determined using standard alignment software such as BLAST. While specific amino acid changes are described above, the invention is intended to encompass mutations to other amino acids aside those specifically described above. In some embodiments, for example, conservative amino acids can be included in place of the mutations set forth above.

Interestingly, the inventors have observed that some naturally occurring PYR/PYL proteins naturally have a P at the position that corresponds to H60. For example, *Arabidopsis* PYL9 has a P at this position. The inventors have found that PYL9 is constitutively active. In some embodiments, a constitutively active PYR/PYL protein is converted to a protein activated by ABA or an ABA agonist by changing a proline at

position "H60" (with reference to the position in *Arabidopsis* PYR1) to a Histidine or other non-proline amino acid.

Accordingly, the present invention provides for PYR/PYL polypeptides that are constitutively active and having a mutation as described above. In some embodiments, the constitutive polypeptides will comprise one or more of the above-described consensus sequences and/or will be substantially identical to one of SEQ ID NOs:2-90.

VI. Use of PYR/PYL Nucleic Acids and Polypeptides of the Invention

The invention provides methods of modulating ABA sensitivity in a plant by altering PYR/PYL expression or activity, for example, by introducing into a plant a recombinant expression cassette comprising a regulatory element (e.g., a promoter) operably linked to a PYR/PYL polynucleotide, i.e., a nucleic acid encoding PYR/PYL or a sequence comprising a portion of the sequence of a PYR/PYL mRNA or complement thereof.

In some embodiments, the methods of the invention comprise increasing and/or ectopically expressing one or more PYR/PYL polynucleotide encoding a PYR/PYL polypeptide in a plant. Such embodiments are useful for increasing ABA sensitivity of a plant, and resulting in, for example, improved stress (e.g., drought) tolerance and/or delayed seed germination (to avoid pre-mature germination, for example as can occur in humid environments or due to other exposure to moisture). For stress tolerance, promoters can be selected that are generally constitutive and are expressed in most plant tissues, or can be leaf or root specific. To affect seed germination, promoters are generally used that result in expression in seed or, in some embodiments, floral organs or embryos.

In some embodiments, the methods of the invention comprise decreasing endogenous PYR/PYL expression in plant, thereby decreasing ABA sensitivity in the plant. Such methods can involve, for example, mutagenesis (e.g., chemical, radiation, transposon or other mutagenesis) of PYR/PYL sequences in a plant to reduce PYR/PYL expression or activity, or introduction of a polynucleotide substantially identical to at least a portion of a PYR/PYL cDNA sequence or a 40 complement thereof (e.g., an "RNAi construct") to reduce PYR/PYL expression. Decreased (or increased) PYR/PYL expression can be used to control the development of abscission zones in leaf petioles and thereby control leaf loss, i.e., delay leaf loss if expression is decreased and speed leaf loss if expression is increased in abscission zones in a leaf.

A. Increasing PYR/PYL Expression or Activity

Isolated sequences prepared as described herein can also be used to prepare expression cassettes that enhance or increase PYR/PYL gene expression. Where overexpression 50 of a gene is desired, the desired gene (or at least the polynucleotide encoding a PYR/PYL polypeptide) from the same species or a different species (or substantially identical to the gene or polynucleotide encoding a PYR/PYL polypeptide from another species) may be used. In some embodiments, to 55 decrease potential sense suppression effects, a polynucleotide encoding a PYR/PYL polypeptide from a different species (or substantially identical to the gene or polynucleotide encoding a PYR/PYL polypeptide from another species) may be used.

Any of a number of means well known in the art can be used to increase PYR/PYL activity in plants. Any organ or plant part can be targeted, such as shoot vegetative organs/structures (e.g. leaves, stems and tubers), roots, flowers and floral organs/structures (e.g. bracts, sepals, petals, stamens, 65 carpels, anthers and ovules), seed (including embryo, endosperm, and seed coat), fruit, abscission zone, etc. Alter-

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natively, one or several PYR/PYL genes can be expressed constitutively (e.g., using the CaMV 35S promoter or other constitutive promoter).

One of skill will recognize that the polypeptides encoded by the genes of the invention, like other proteins, have different domains which perform different functions. Thus, the overexpressed or ectopically expressed polynucleotide sequences need not be full length, so long as the desired functional domain of the protein is expressed. Alternatively, or in addition, active PYR/PYL proteins can be expressed as fusions, without necessarily significantly altering PYR/PYL activity. Examples of fusion partners include, but are not limited to, poly-His or other tag sequences.

B. Decreasing PYR/PYL Expression or Activity

A number of methods can be used to inhibit gene expression in plants. A variety of methods to inhibit gene expression are known and can be used to inhibit expression of one of more PYR/PYL genes. See, e.g., U.S. Pat. Nos. 5,759,829; 5,107,065; 5,231,020; 5,283,184; 6,506,559; 6,573,099, 6.326,193; 7.109,393. For instance, antisense technology can be conveniently used. To accomplish this, a nucleic acid segment from the desired gene is cloned and operably linked to a promoter such that the antisense strand of RNA will be transcribed. The expression cassette is then transformed into plants and the antisense strand of RNA is produced. In plant cells, it has been suggested that antisense RNA inhibits gene expression by preventing the accumulation of mRNA which encodes the enzyme of interest, see, e.g., Sheehy et al., Proc. Nat. Acad. Sci. USA, 85:8805-8809 (1988); Pnueli et al., The Plant Cell 6:175-186 (1994); and Hiatt et al., U.S. Pat. No. 4,801,340.

The antisense nucleic acid sequence transformed into plants will be substantially identical to at least a portion of the endogenous gene or genes to be repressed. The sequence, however, does not have to be perfectly identical to inhibit expression. Thus, an antisense or sense nucleic acid molecule encoding only a portion of PYR/PYL polypeptide, or a portion of the PYR/PYL cDNA, can be useful for producing a plant in which PYR/PYL expression is suppressed. The vectors of the present invention can be designed such that the inhibitory effect applies to other proteins within a family of genes exhibiting homology or substantial homology to the target gene. In some embodiments, it may be desirable to inhibit the expression of more than one PYR/PYL polypeptide at the same time using one or more antisense or sense or other siRNA nucleic acid molecules.

For antisense suppression, the introduced sequence also need not be full length relative to either the primary transcription product or fully processed mRNA. Generally, higher homology can be used to compensate for the use of a shorter sequence. Furthermore, the introduced sequence need not have the same intron or exon pattern, and homology of noncoding segments may be equally effective. For example, a sequence of between about 30 or 40 nucleotides can be used, and in some embodiments, about full length nucleotides should be used, though a sequence of at least about 20, 50 100, 200, or 500 nucleotides can be used.

Catalytic RNA molecules or ribozymes can also be used to inhibit expression of PYR/PYL genes. It is possible to design ribozymes that specifically pair with virtually any target RNA and cleave the phosphodiester backbone at a specific location, thereby functionally inactivating the target RNA. In carrying out this cleavage, the ribozyme is not itself altered, and is thus capable of recycling and cleaving other molecules, making it a true enzyme. The inclusion of ribozyme sequences within antisense RNAs confers RNA-cleaving activity upon them, thereby increasing the activity of the constructs.

A number of classes of ribozymes have been identified. One class of ribozymes is derived from a number of small circular RNAs that are capable of self-cleavage and replication in plants. The RNAs replicate either alone (viroid RNAs) or with a helper virus (satellite RNAs). Examples include 5 RNAs from avocado sunblotch viroid and the satellite RNAs from tobacco ringspot virus, lucerne transient streak virus, velvet tobacco mottle virus, *solanum* nodiflorum mottle virus and subterranean clover mottle virus. The design and use of target RNA-specific ribozymes is described in Haseloff et al. 10 *Nature*, 334:585-591 (1988).

Another method of suppression is sense suppression (also known as co-suppression). Introduction of expression cassettes in which a nucleic acid is configured in the sense orientation with respect to the promoter has been shown to be 15 an effective means by which to block the transcription of target genes. For an example of the use of this method to modulate expression of endogenous genes see, Napoli et al., *The Plant Cell* 2:279-289 (1990); Flavell, *Proc. Natl. Acad. Sci., USA* 91:3490-3496 (1994); Kooter and Mol, *Current* 20 *Opin. Biol.* 4:166-171 (1993); and U.S. Pat. Nos. 5,034,323, 5,231,020, and 5,283,184.

Generally, where inhibition of expression is desired, some transcription of the introduced sequence occurs. The effect may occur where the introduced sequence contains no coding 25 sequence per se, but only intron or untranslated sequences homologous to sequences present in the primary transcript of the endogenous sequence. The introduced sequence generally will be substantially identical to the endogenous sequence intended to be repressed. This minimal identity will typically be greater than about 65%, but a higher identity can exert a more effective repression of expression of the endogenous sequences. In some embodiments, sequences with substantially greater identity are used, e.g., at least about 80, at least about 95%, or 100% identity are used. As with antisense 35 regulation, the effect can be designed and tested to apply to any other proteins within a similar family of genes exhibiting homology or substantial homology.

For sense suppression, the introduced sequence in the expression cassette, needing less than absolute identity, also 40 need not be full length, relative to either the primary transcription product or fully processed mRNA. This may be preferred to avoid concurrent production of some plants that are overexpressers. A higher identity in a shorter than full length sequence compensates for a longer, less identical 45 sequence. Furthermore, the introduced sequence need not have the same intron or exon pattern, and identity of noncoding segments will be equally effective. In some embodiments, a sequence of the size ranges noted above for antisense regulation is used, i.e., 30-40, or at least about 20, 50, 100, 50 200, 500 or more nucleotides.

Endogenous gene expression may also be suppressed by means of RNA interference (RNAi) (and indeed co-suppression can be considered a type of RNAi), which uses a doublestranded RNA having a sequence identical or similar to the 55 sequence of the target gene. RNAi is the phenomenon in which when a double-stranded RNA having a sequence identical or similar to that of the target gene is introduced into a cell, the expressions of both the inserted exogenous gene and target endogenous gene are suppressed. The double-stranded 60 RNA may be formed from two separate complementary RNAs or may be a single RNA with internally complementary sequences that form a double-stranded RNA. Although complete details of the mechanism of RNAi are still unknown, it is considered that the introduced double-stranded 65 RNA is initially cleaved into small fragments, which then serve as indexes of the target gene in some manner, thereby

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degrading the target gene. RNAi is known to be also effective in plants (see, e.g., Chuang, C. F. & Meyerowitz, E. M., *Proc.* Natl. Acad. Sci. USA 97: 4985 (2000); Waterhouse et al., Proc. Natl. Acad. Sci. USA 95:13959-13964 (1998); Tabara et al. Science 282:430-431 (1998); Matthew, Comp Funct Genom 5: 240-244 (2004); Lu, et al., Nucleic Acids Research 32(21):e171 (2004)). For example, to achieve suppression of the expression of a DNA encoding a protein using RNAi, a double-stranded RNA having the sequence of a DNA encoding the protein, or a substantially similar sequence thereof (including those engineered not to translate the protein) or fragment thereof, is introduced into a plant of interest. The resulting plants may then be screened for a phenotype associated with the target protein and/or by monitoring steadystate RNA levels for transcripts encoding the protein. Although the genes used for RNAi need not be completely identical to the target gene, they may be at least 70%, 80%, 90%, 95% or more identical to the target (e.g., PYR/PYL) gene sequence. See, e.g., U.S. Patent Publication No. 2004/ 0029283. The constructs encoding an RNA molecule with a stem-loop structure that is unrelated to the target gene and that is positioned distally to a sequence specific for the gene of interest may also be used to inhibit target gene expression. See, e.g., U.S. Patent Publication No. 2003/0221211.

The RNAi polynucleotides can encompass the full-length target RNA or may correspond to a fragment of the target RNA. In some cases, the fragment will have fewer than 100, 200, 300, 400, 500 600, 700, 800, 900 or 1,000 nucleotides corresponding to the target sequence. In addition, in some embodiments, these fragments are at least, e.g., 50, 100, 150, 200, or more nucleotides in length. In some cases, fragments for use in RNAi will be at least substantially similar to regions of a target protein that do not occur in other proteins in the organism or may be selected to have as little similarity to other organism transcripts as possible, e.g., selected by comparison to sequences in analyzing publicly-available sequence databases.

Expression vectors that continually express siRNA in transiently- and stably-transfected have been engineered to express small hairpin RNAs, which get processed in vivo into siRNAs molecules capable of carrying out gene-specific silencing (Brummelkamp et al., *Science* 296:550-553 (2002), and Paddison, et al., *Genes & Dev.* 16:948-958 (2002)). Post-transcriptional gene silencing by double-stranded RNA is discussed in further detail by Hammond et al. *Nature Rev Gen* 2: 110-119 (2001), Fire et al. *Nature* 391: 806-811 (1998) and Timmons and Fire *Nature* 395: 854 (1998).

One of skill in the art will recognize that using technology based on specific nucleotide sequences (e.g., antisense or sense suppression technology), families of homologous genes can be suppressed with a single sense or antisense transcript. For instance, if a sense or antisense transcript is designed to have a sequence that is conserved among a family of genes, then multiple members of a gene family can be suppressed. Conversely, if the goal is to only suppress one member of a homologous gene family, then the sense or antisense transcript should be targeted to sequences with the most variance between family members.

Another means of inhibiting PYR/PYL function in a plant is by creation of dominant negative mutations. In this approach, non-functional, mutant PYR/PYL polypeptides, which retain the ability to interact with wild-type subunits are introduced into a plant. A dominant negative construct also can be used to suppress PYR/PYL expression in a plant. A dominant negative construct useful in the invention generally contains a portion of the complete PYR/PYL coding sequence sufficient, for example, for DNA-binding or for a

protein-protein interaction such as a homodimeric or heterodimeric protein-protein interaction but lacking the transcriptional activity of the wild type protein.

#### VII. Recombinant Expression Vectors

Once the coding or cDNA sequence for PYR/PYL is 5 obtained, it can also be used to prepare an expression cassette for expressing the PYR/PYL protein in a transgenic plant, directed by a heterologous promoter. Increased expression of PYR/PYL polynucleotide is useful, for example, to produce plants with enhanced drought-resistance. Alternatively, as described above, expression vectors can also be used to express PYR/PYL polynucleotides and variants thereof that inhibit endogenous PYR/PYL expression.

Any of a number of means well known in the art can be used to increase or decrease PYR/PYL activity or expression in plants. Any organ can be targeted, such as shoot vegetative organs/structures (e.g. leaves, stems and tubers), roots, flowers and floral organs/structures (e.g. bracts, sepals, petals, stamens, carpels, anthers and ovules), seed (including 20 embryo, endosperm, and seed coat) and fruit. Alternatively, the PYR/PYL gene can be expressed constitutively (e.g., using the CaMV 35S promoter).

To use PYR/PYL coding or cDNA sequences in the above techniques, recombinant DNA vectors suitable for transformation of plant cells are prepared. Techniques for transforming a wide variety of higher plant species are well known and described in the technical and scientific literature. See, e.g., Weising et al. *Ann. Rev. Genet.* 22:421-477 (1988). A DNA sequence coding for the PYR/PYL polypeptide preferably will be combined with transcriptional and translational initiation regulatory sequences which will direct the transcription of the sequence from the gene in the intended tissues of the transformed plant.

For example, a plant promoter fragment may be employed 35 to direct expression of the PYR/PYL gene in all tissues of a regenerated plant. Such promoters are referred to herein as "constitutive" promoters and are active under most environmental conditions and states of development or cell differentiation. Examples of constitutive promoters include the cauliflower mosaic virus (CaMV) 35S transcription initiation region, the 1'- or 2'-promoter derived from T-DNA of *Agrobacterium tumafaciens*, and other transcription initiation regions from various plant genes known to those of skill.

Alternatively, the plant promoter may direct expression of the PYR/PYL protein in a specific tissue (tissue-specific promoters) or may be otherwise under more precise environmental control (inducible promoters). Examples of tissue-specific promoters under developmental control include promoters that initiate transcription only in certain tissues, such as leaves or guard cells (including but not limited to those described in WO/2005/085449; U.S. Pat. No. 6,653,535; Li et al., *Sci China C Life Sci.* 2005 April; 48(2):181-6; Husebye, et al., *Plant Physiol*, April 2002, Vol. 128, pp. 1180-1188; and Plesch, et al., *Gene*, Volume 249, Number 1, 16 May 2000, pp. 55 83-89(7)). Examples of environmental conditions that may affect transcription by inducible promoters include anaerobic conditions, elevated temperature, or the presence of light.

If proper protein expression is desired, a polyadenylation region at the 3'-end of the coding region should be included. 60 The polyadenylation region can be derived from the natural gene, from a variety of other plant genes, or from T-DNA.

The vector comprising the sequences (e.g., promoters or PYR/PYL coding regions) will typically comprise a marker gene that confers a selectable phenotype on plant cells. For 65 example, the marker may encode biocide resistance, particularly antibiotic resistance, such as resistance to kanamycin,

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G418, bleomycin, hygromycin, or herbicide resistance, such as resistance to chlorosluforon or Basta.

In some embodiments, the PYR/PYL nucleic acid sequence is expressed recombinantly in plant cells to enhance and increase levels of total PYR/PYL polypeptide. A variety of different expression constructs, such as expression cassettes and vectors suitable for transformation of plant cells can be prepared. Techniques for transforming a wide variety of higher plant species are well known and described in the technical and scientific literature. See, e.g., Weising et al. *Ann. Rev. Genet.* 22:421-477 (1988). A DNA sequence coding for a PYR/PYL protein can be combined with cis-acting (promoter) and trans-acting (enhancer) transcriptional regulatory sequences to direct the timing, tissue type and levels of transcription in the intended tissues of the transformed plant. Translational control elements can also be used.

The invention provides a PYR/PYL nucleic acid operably linked to a promoter which, in some embodiments, is capable of driving the transcription of the PYR/PYL coding sequence in plants. The promoter can be, e.g., derived from plant or viral sources. The promoter can be, e.g., constitutively active, inducible, or tissue specific. In construction of recombinant expression cassettes, vectors, transgenics, of the invention, a different promoters can be chosen and employed to differentially direct gene expression, e.g., in some or all tissues of a plant or animal.

#### A. Constitutive Promoters

A promoter fragment can be employed to direct expression of a PYR/PYL nucleic acid in all transformed cells or tissues, e.g., as those of a regenerated plant. The term "constitutive regulatory element" means a regulatory element that confers a level of expression upon an operatively linked nucleic molecule that is relatively independent of the cell or tissue type in which the constitutive regulatory element is expressed. A constitutive regulatory element that is expressed in a plant generally is widely expressed in a large number of cell and tissue types. Promoters that drive expression continuously under physiological conditions are referred to as "constitutive" promoters and are active under most environmental conditions and states of development or cell differentiation.

A variety of constitutive regulatory elements useful for ectopic expression in a transgenic plant are well known in the art. The cauliflower mosaic virus 35S (CaMV 35S) promoter, for example, is a well-characterized constitutive regulatory element that produces a high level of expression in all plant tissues (Odell et al., Nature 313:810-812 (1985)). The CaMV 35S promoter can be particularly useful due to its activity in numerous diverse plant species (Benfey and Chua, Science 250:959-966 (1990); Futterer et al., Physiol. Plant 79:154 (1990); Odell et al., supra, 1985). A tandem 35S promoter, in which the intrinsic promoter element has been duplicated, confers higher expression levels in comparison to the unmodified 35S promoter (Kay et al., Science 236:1299 (1987)). Other useful constitutive regulatory elements include, for example, the cauliflower mosaic virus 19S promoter; the Figwort mosaic virus promoter; and the nopaline synthase (nos) gene promoter (Singer et al., *Plant Mol. Biol.* 14:433 (1990); An, Plant Physiol. 81:86 (1986)).

Additional constitutive regulatory elements including those for efficient expression in monocots also are known in the art, for example, the pEmu promoter and promoters based on the rice Actin-1 5' region (Last et al., *Theor. Appl. Genet.* 81:581 (1991); Mcelroy et al., *Mol. Gen. Genet.* 231:150 (1991); Mcelroy et al., *Plant Cell* 2:163 (1990)). Chimeric regulatory elements, which combine elements from different genes, also can be useful for ectopically expressing a nucleic

acid molecule encoding a PYR/PYL protein (Comai et al., *Plant Mol. Biol.* 15:373 (1990)).

Other examples of constitutive promoters include the 1'- or 2'-promoter derived from T-DNA of Agrobacterium tumafaciens (see, e.g., Mengiste (1997) supra; O'Grady (1995) 5 Plant Mol. Biol. 29:99-108); actin promoters, such as the Arabidopsis actin gene promoter (see, e.g., Huang (1997) Plant Mol. Biol. 1997 33:125-139); alcohol dehydrogenase (Adh) gene promoters (see, e.g., Millar (1996) *Plant Mol*. Biol. 31:897-904); ACT11 from Arabidopsis (Huang et al. 10 Plant Mol. Biol. 33:125-139 (1996)), Cat3 from Arabidopsis (GenBank No. U43147, Zhong et al., Mol. Gen. Genet. 251: 196-203 (1996)), the gene encoding stearoyl-acyl carrier protein desaturase from Brassica napus (Genbank No. X74782, Solocombe et al. Plant Physiol. 104:1167-1176 (1994)), 15 Gpc1 from maize (GenBank No. X15596, Martinez et al. J. Mol. Biol. 208:551-565 (1989)), Gpc2 from maize (GenBank No. U45855, Manjunath et al., Plant Mol. Biol. 33:97-112 (1997)), other transcription initiation regions from various plant genes known to those of skill. See also Holtorf *Plant* 20 Mol. Biol. 29:637-646 (1995).

## B. Inducible Promoters

Alternatively, a plant promoter may direct expression of the PYR/PYL gene under the influence of changing environmental conditions or developmental conditions. Examples of 25 environmental conditions that may effect transcription by inducible promoters include anaerobic conditions, elevated temperature, drought, or the presence of light. Such promoters are referred to herein as "inducible" promoters. For example, the invention can incorporate drought-specific promoter such as the drought-inducible promoter of maize (Busk (1997) supra); or alternatively the cold, drought, and high salt inducible promoter from potato (Kirch (1997) *Plant Mol. Biol.* 33:897-909).

Alternatively, plant promoters which are inducible upon 35 exposure to plant hormones, such as auxins, are used to express the PYR/PYL gene. For example, the invention can use the auxin-response elements El promoter fragment (AuxREs) in the soybean (*Glycine max* L.) (Liu (1997) *Plant Physiol.* 115:397-407); the auxin-responsive *Arabidopsis* 40 GST6 promoter (also responsive to salicylic acid and hydrogen peroxide) (Chen (1996) *Plant J.* 10: 955-966); the auxin-inducible parC promoter from tobacco (Sakai (1996) 37:906-913); a plant biotin response element (Streit (1997) *Mol. Plant. Microbe Interact.* 10:933-937); and, the promoter 45 responsive to the stress hormone abscisic acid (Sheen (1996) *Science* 274:1900-1902).

Plant promoters inducible upon exposure to chemicals reagents that may be applied to the plant, such as herbicides or antibiotics, are also useful for expressing the PYR/PYL gene. 50 For example, the maize In2-2 promoter, activated by benzenesulfonamide herbicide safeners, can be used (De Veylder (1997) Plant Cell Physiol. 38:568-577); application of different herbicide safeners induces distinct gene expression patterns, including expression in the root, hydathodes, and the 55 shoot apical meristem. A PYR/PYL coding sequence can also be under the control of, e.g., a tetracycline-inducible promoter, e.g., as described with transgenic tobacco plants containing the Avena sativa L. (oat) arginine decarboxylase gene (Masgrau (1997) Plant J. 11:465-473); or, a salicylic acid- 60 responsive element (Stange (1997) Plant J. 11:1315-1324; Uknes et al., *Plant Cell* 5:159-169 (1993); Bi et al., *Plant J.* 8:235-245 (1995)).

Examples of useful inducible regulatory elements include copper-inducible regulatory elements (Mett et al., *Proc. Natl. Acad. Sci. USA* 90:4567-4571 (1993); Furst et al., *Cell* 55:705-717 (1988)); tetracycline and chlor-tetracycline-in-

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ducible regulatory elements (Gatz et al., Plant J. 2:397-404 (1992); Roder et al., Mol. Gen. Genet. 243:32-38 (1994); Gatz, Meth. Cell Biol. 50:411-424 (1995)); ecdysone inducible regulatory elements (Christopherson et al., Proc. Natl. Acad. Sci. USA 89:6314-6318 (1992); Kreutzweiser et al., Ecotoxicol. Environ. Safety 28:14-24 (1994)); heat shock inducible regulatory elements (Takahashi et al., Plant Physiol. 99:383-390 (1992); Yabe et al., Plant Cell Physiol. 35:1207-1219 (1994); Ueda et al., Mol. Gen. Genet. 250:533-539 (1996)); and lac operon elements, which are used in combination with a constitutively expressed lac repressor to confer, for example, IPTG-inducible expression (Wilde et al., EMBO J. 11:1251-1259 (1992)). An inducible regulatory element useful in the transgenic plants of the invention also can be, for example, a nitrate-inducible promoter derived from the spinach nitrite reductase gene (Back et al., Plant Mol.

Biol. 17:9 (1991)) or a light-inducible promoter, such as that associated with the small subunit of RuBP carboxylase or the LHCP gene families (Feinbaum et al., *Mol. Gen. Genet.* 226:449 (1991); Lam and Chua, *Science* 248:471 (1990)).

### C. Tissue-Specific Promoters

Alternatively, the plant promoter may direct expression of the PYR/PYL gene in a specific tissue (tissue-specific promoters). Tissue specific promoters are transcriptional control elements that are only active in particular cells or tissues at specific times during plant development, such as in vegetative tissues or reproductive tissues.

Examples of tissue-specific promoters under developmental control include promoters that initiate transcription only (or primarily only) in certain tissues, such as vegetative tissues, e.g., roots or leaves, or reproductive tissues, such as fruit, ovules, seeds, pollen, pistols, flowers, or any embryonic tissue, or epidermis or mesophyll. Reproductive tissue-specific promoters may be, e.g., ovule-specific, embryo-specific, endosperm-specific, integument-specific, seed and seed coat-specific, pollen-specific, petal-specific, sepal-specific, or some combination thereof. In some embodiments, the promoter is cell-type specific, e.g., guard cell-specific.

Other tissue-specific promoters include seed promoters. Suitable seed-specific promoters are derived from the following genes: MAC1 from maize (Sheridan (1996) *Genetics* 142:1009-1020); Cat3 from maize (GenBank No. L05934, Abler (1993) *Plant Mol. Biol.* 22:10131-1038); vivparous-1 from *Arabidopsis* (Genbank No. U93215); atmyc1 from *Arabidopsis* (Urao (1996) *Plant Mol. Biol.* 32:571-57; Conceicao (1994) *Plant* 5:493-505); napA from *Brassica napus* (GenBank No. J02798, Josefsson (1987) JBL 26:12196-1301); and the napin gene family from *Brassica napus* (Sjodahl (1995) *Planta* 197:264-271).

A variety of promoters specifically active in vegetative tissues, such as leaves, stems, roots and tubers, can also be used to express polynucleotides encoding PYR/PYL polypeptides (or RNAi or antisense or sense constructs). For example, promoters controlling patatin, the major storage protein of the potato tuber, can be used, see, e.g., Kim (1994) Plant Mol. Biol. 26:603-615; Martin (1997) Plant J. 11:53-62. The ORF13 promoter from Agrobacterium rhizogenes that exhibits high activity in roots can also be used (Hansen (1997) Mol. Gen. Genet. 254:337-343. Other useful vegetative tissue-specific promoters include: the tarin promoter of the gene encoding a globulin from a major taro (Colocasia esculenta L. Schott) corm protein family, tarin (Bezerra (1995) Plant Mol. Biol. 28:137-144); the curculin promoter active during taro corm development (de Castro (1992) Plant Cell 4:1549-1559) and the promoter for the tobacco root-

specific gene TobRB7, whose expression is localized to root meristem and immature central cylinder regions (Yamamoto (1991) *Plant Cell* 3:371-382).

Leaf-specific promoters, such as the ribulose biphosphate carboxylase (RBCS) promoters can be used. For example, the 5 tomato RBCS1, RBCS2 and RBCS3A genes are expressed in leaves and light-grown seedlings, only RBCS1 and RBCS2 are expressed in developing tomato fruits (Meier (1997) FEBS Lett. 415:91-95). A ribulose bisphosphate carboxylase promoters expressed almost exclusively in mesophyll cells in 10 leaf blades and leaf sheaths at high levels, described by Matsuoka (1994) Plant J. 6:311-319, can be used. Another leafspecific promoter is the light harvesting chlorophyll a/b binding protein gene promoter, see, e.g., Shiina (1997) Plant Physiol. 115:477-483; Casal (1998) Plant Physiol. 116:1533- 15 1538. The Arabidopsis thaliana myb-related gene promoter (Atmyb5) described by Li (1996) FEBS Lett. 379:117-121, is leaf-specific. The Atmyb5 promoter is expressed in developing leaf trichomes, stipules, and epidermal cells on the margins of young rosette and cauline leaves, and in immature 20 seeds. Atmyb5 mRNA appears between fertilization and the 16 cell stage of embryo development and persists beyond the heart stage. A leaf promoter identified in maize by Busk (1997) *Plant J.* 11:1285-1295, can also be used.

Another class of useful vegetative tissue-specific promot- 25 ers are meristematic (root tip and shoot apex) promoters. For example, the "SHOOTMERISTEMLESS" and "SCARE-CROW" promoters, which are active in the developing shoot or root apical meristems, described by Di Laurenzio (1996) Cell 86:423-433; and, Long (1996) Nature 379:66-69; can be 30 used. Another useful promoter is that which controls the expression of 3-hydroxy-3-methylglutaryl coenzyme A reductase HMG2 gene, whose expression is restricted to meristematic and floral (secretory zone of the stigma, mature pollen grains, gynoecium vascular tissue, and fertilized 35 ovules) tissues (see, e.g., Enjuto (1995) Plant Cell. 7:517-527). Also useful are kn1-related genes from maize and other species which show meristem-specific expression, see, e.g., Granger (1996) Plant Mol. Biol. 31:373-378; Kerstetter (1994) Plant Cell 6:1877-1887; Hake (1995) Philos. Trans. 40 R. Soc. Lond. B. Biol. Sci. 350:45-51. For example, the Arabidopsis thaliana KNAT1 promoter (see, e.g., Lincoln (1994) Plant Cell 6:1859-1876).

One of skill will recognize that a tissue-specific promoter may drive expression of operably linked sequences in tissues 45 other than the target tissue. Thus, as used herein a tissue-specific promoter is one that drives expression preferentially in the target tissue, but may also lead to some expression in other tissues as well.

In another embodiment, the PYR/PYL polynucleotide is 50 expressed through a transposable element. This allows for constitutive, yet periodic and infrequent expression of the constitutively active polypeptide. The invention also provides for use of tissue-specific promoters derived from viruses including, e.g., the tobamovirus subgenomic promoter 55 (Kumagai (1995) *Proc. Natl. Acad. Sci. USA* 92:1679-1683; the rice tungro bacilliform virus (RTBV), which replicates only in phloem cells in infected rice plants, with its promoter which drives strong phloem-specific reporter gene expression; the cassaya vein mosaic virus (CVMV) promoter, with 60 highest activity in vascular elements, in leaf mesophyll cells, and in root tips (Verdaguer (1996) *Plant Mol. Biol.* 31:1129-1139).

VIII. Production of Transgenic Plants

As detailed herein, the present invention provides for transgenic plants comprising recombinant expression cassettes either for expressing PYR/PYL proteins in a plant or for 30

inhibiting or reducing endogenous PYR/PYL expression. Thus, in some embodiments, a transgenic plant is generated that contains a complete or partial sequence of an endogenous PYR/PYL encoding polynucleotide, either for increasing or reducing PYR/PYL expression and activity. In some embodiments, a transgenic plant is generated that contains a complete or partial sequence of a polynucleotide that is substantially identical to an endogenous PYR/PYL encoding polynucleotide, either for increasing or reducing PYR/PYL expression and activity. In some embodiments, a transgenic plant is generated that contains a complete or partial sequence of a polynucleotide that is from a species other than the species of the transgenic plant. It should be recognized that transgenic plants encompass the plant or plant cell in which the expression cassette is introduced as well as progeny of such plants or plant cells that contain the expression cassette, including the progeny that have the expression cassette stably integrated in a chromosome.

A recombinant expression vector comprising a PYR/PYL coding sequence driven by a heterologous promoter may be introduced into the genome of the desired plant host by a variety of conventional techniques. For example, the DNA construct may be introduced directly into the genomic DNA of the plant cell using techniques such as electroporation and microinjection of plant cell protoplasts, or the DNA construct can be introduced directly to plant tissue using ballistic methods, such as DNA particle bombardment. Alternatively, the DNA construct may be combined with suitable T-DNA flanking regions and introduced into a conventional Agrobacterium tumefaciens host vector. The virulence functions of the Agrobacterium tumefaciens host will direct the insertion of the construct and adjacent marker into the plant cell DNA when the cell is infected by the bacteria. While transient expression of PYR/PYL is encompassed by the invention, generally expression of construction of the invention will be from insertion of expression cassettes into the plant genome, e.g., such that at least some plant offspring also contain the integrated expression cassette.

Microinjection techniques are also useful for this purpose. These techniques are well known in the art and thoroughly described in the literature. The introduction of DNA constructs using polyethylene glycol precipitation is described in Paszkowski et al. *EMBO J.* 3:2717-2722 (1984). Electroporation techniques are described in Fromm et al. *Proc. Natl. Acad. Sci. USA* 82:5824 (1985). Ballistic transformation techniques are described in Klein et al. *Nature* 327:70-73 (1987).

Agrobacterium tumefaciens-mediated transformation techniques, including disarming and use of binary vectors, are well described in the scientific literature. See, for example, Horsch et al. Science 233:496-498 (1984), and Fraley et al. Proc. Natl. Acad. Sci. USA 80:4803 (1983).

Transformed plant cells derived by any of the above transformation techniques can be cultured to regenerate a whole plant that possesses the transformed genotype and thus the desired phenotype such as enhanced drought-resistance. Such regeneration techniques rely on manipulation of certain phytohormones in a tissue culture growth medium, typically relying on a biocide and/or herbicide marker which has been introduced together with the desired nucleotide sequences. Plant regeneration from cultured protoplasts is described in Evans et al., Protoplasts Isolation and Culture, Handbook of Plant Cell Culture, pp. 124-176, MacMillilan Publishing Company, New York, 1983; and Binding, Regeneration of Plants, Plant Protoplasts, pp. 21-73, CRC Press, Boca Raton, 1985. Regeneration can also be obtained from plant callus,

explants, organs, or parts thereof. Such regeneration techniques are described generally in Klee et al. *Ann. Rev. of Plant Phys.* 38:467-486 (1987).

One of skill will recognize that after the expression cassette is stably incorporated in transgenic plants and confirmed to be operable, it can be introduced into other plants by sexual crossing. Any of a number of standard breeding techniques can be used, depending upon the species to be crossed.

The expression cassettes of the invention can be used to confer drought resistance on essentially any plant. Thus, the invention has use over a broad range of plants, including species from the genera Asparagus, Atropa, Avena, Brassica, Citrus, Citrullus, Capsicum, Cucumis, Cucurbita, Daucus, Fragaria, Glycine, Gossypium, Helianthus, Heterocallis, Hordeum, Hyoscyamus, Lactuca, Linum, Lolium, Lycopersicon, Malus, Manihot, Majorana, Medicago, Nicotiana, Oryza, Panieum, Pannesetum, Persea, Pisum, Pyrus, Prunus, Raphanus, Secale, Senecio, Sinapis, Solanum, Sorghum, Trigonella, Triticum, Vitis, Vigna, and, Zea. In some embodiments, the plant is selected from the group consisting of rice, maize, wheat, soybeans, cotton, canola, turfgrass, and alfalfa. In some embodiments, the plant is an ornamental plant. In some embodiment, the plant is a vegetable- or fruit-producing plant.

Those of skill will recognize that a number of plant species 25 can be used as models to predict the phenotypic effects of transgene expression in other plants. For example, it is well recognized that both tobacco (Nicotiana) and *Arabidopsis* plants are useful models of transgene expression, particularly in other dicots.

The plants of the invention have either enhanced or reduced abscisic acid sensitivity compared to plants are otherwise identical except for expression of PYR/PYL. Abscisic acid sensitivity can be monitored by observing or measuring any phenotype mediated by ABA. Those of skill in the art will recognize that ABA is a well-studied plant hormone and that ABA mediates many changes in characteristics, any of which can be monitored to determined whether ABA sensitivity has been modulated. In some embodiments, modulated ABA sensitivity is manifested by altered timing of seed germination or altered stress (e.g., drought) tolerance.

Drought resistance can assayed according to any of a number of well-known techniques. For example, plants can be grown under conditions in which less than optimum water is provided to the plant. Drought resistance can be determined by any of a number of standard measures including turgor pressure, growth, yield, and the like. In some embodiments, the methods described in the Example section, below can be conveniently used.

## **EXAMPLES**

The following examples are offered to illustrate, but not to limit the claimed invention.

# Example 1

## PYR/PYL Modulation of ABA Signaling

Unlike biochemical screens for ABA-binding proteins, 60 genetic analyses focused on ABA perception have not yet identified proteins resembling receptors, suggesting that the receptor(s) may be functionally redundant, have overlapping functions or cannot mutate to yield viable gametes or seedlings (P. McCourt, *Annual Review of Plant Physiology and 65 Plant Molecular Biology* 50, 219 (1999)). As a complementary approach, we have pursued a chemical genetic strategy in

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plants (Y. Zhao et al., *Nat Chem Biol* 3, 716 (2007)). This approach can be advantageous for organisms with highly redundant genomes, because the variable selectivity of small molecules can cause phenotypes not revealed by single gene mutations (N. Raikhel, M. Pirrung, *PLANT PHYSIOLOGY* 138, 563 (2005); S. Cutler, P. McCourt, *Plant Physiol.* 138, 558 (2005)). For example an antagonist with low selectivity can perturb the function of an entire protein family (as seen with microtubule antagonists), while an agonist with high selectivity may illuminate the function of an individual member of normally redundant receptors, as we describe here with pyrabactin 3 (FIG. 1A).

Pyrabactin is a Seed-selective ABA Agonist

As part of an earlier effort, we identified a germination inhibitor named pyrabactin (Y. Zhao et al., Nat Chem Biol 3, 716 (2007)). By examining the sensitivity of multiple wild accessions to pyrabactin, we found that the Cold Spring Harbor Lab wild type, which is ABA-hypersensitive and hyperdormant, is also hypersensitive to pyrabactin, but not an inactive analog apyrabactin 4 (FIG. 1A). This suggested that pyrabactin might act through the ABA response pathway. To test this hypothesis, we examined the pyrabactin sensitivity of mutant lines with altered ABA signaling, biosynthesis or gibberellic acid (GA) perception. We found that ABA perception, but not biosynthesis, mutants affect pyrabactin sensitivity (FIG. 1B). Additionally an rgl2-1 mutant line, which does not require GA during germination (S. Lee et al., Genes Dev. 16, 646 (Mar. 1, 2002, 2002)), has normal pyrabactin sensitivity (FIG. 1B). Together, these observations suggest that pyrabactin inhibits germination by activating the ABA signaling pathway, rather than by modulating ABA or GA biosynthesis.

We next performed microarray experiments to evaluate the similarity of the transcriptional responses induced by ABA and pyrabactin treatments. For microarray, tissue was prepared and RNA extracted from Columbia wild type seeds sown on 0.5×MS media (2500 seeds per 150 mm plate) containing either 1 µM ABA, 25 µM pyrabactin, 25 µM 2,4-Dinitrophenol (DNP), 1 µM cycloheximide, 2 µM methotrexate or 1% DMSO control plates (all chemicals are dissolved in DMSO). The concentrations utilized for these experiments were normalized for germination inhibition activity by dose curve analyses, i.e. the amount of both compounds required to ensure 100% inhibition of germination when scored 3 days post-imbibition. ABA (±stereoisomers), DNP, cycloheximide and methotrexate were purchased from Sigma Aldrich. Seeds were stratified for 4 days and then incubated in the dark at room temperature for 24 hours. Seeds were collected and frozen in liquid nitrogen, then ground to fine powder form 50 with frozen mortar and pestle, after which total RNA was extracted using the RNAqueous kit (Ambion; Austin, USA) for the first set of replicate samples. Subsequent RNA extractions were performed using the phenol-chloroform extraction protocol, as described by (Y. Suzuki, T. Kawazu, H. Koyama, 55 Biotechniques, 37, 542 (October, 2004)). For each sample of total RNA, 1 µl of RNA was quantified in 99 µl 10 mM Tris-Cl (pH 7.4) by the GeneQuant RNA/DNA Calculator (GE Healthcare Bio-Sciences Corp.; New Jersey, USA), where absorbance measurements were taken at 260 nm and 280 nm. Purity of the RNA was assessed by OD<sub>260</sub>/OD<sub>280</sub> ratios (only ratios between 1.7 and 2.2 were used), while quality of the RNA was assessed by gel electrophoresis. Total RNA samples were converted to biotin-labeled cRNA using oligodT priming as described by the manufacturer (Enzo kit; Affymetrix; Santa Clara, USA) and hybridized to 22K ATH1 Affymetrix microarrays at the CAGEF (University of Toronto). Duplicate biological replicate samples were hybrid-

ized for DNP, cycloheximide and methotrexate, triplicate for control and quadruplicate samples were hybridized for, pyrabactin and ABA treatments. Probe sets with expression signals called present or marginal by the statistical algorithms applied to the microarrays as described as described for the 5 GCOS/MAS5.0 algorithm (Affymetrix; Santa Clara, USA). Significance Analysis of Microarrays was used to identify probe sets that are significantly regulated by treatments using unlogged data, with a false discovery rate (FDR) at about 5%. Average transcript levels were compared to control values to compute fold-change, which was in turn log2 transformed and used to compute Pearson Correlation Coefficients between experiments.

We first examined seeds treated with both compounds for 24 hours. Due to inhibitory effects on seedling development, 15 any two germination inhibitors will share some common responses; we therefore used a previously defined set of germination responsive transcripts (G. W. Bassel et al., Plant Physiol 147, 143 (2008)) to minimize developmental effects in our comparisons. 1225 probe sets were identified as 20 responsive to either ABA or pyrabactin using SAM analysis (V. G. Tusher, R. Tibshirani, G. Chu, Proc. Nat'l. Acad. Sci. USA 98, 5116 (2001)), after removal of 403 germinationregulated transcripts. Scatter plots comparing a probe's responsiveness to pyrabactin and ABA demonstrate highly 25 correlated responses (r=0.98; FIG. 1C), consistent with the hypothesis that pyrabactin activates ABA signaling. As a control, we also profiled the effects of the three germination inhibitors (G. W. Bassel et al., *Plant Physiol* 147, 143 (2008)) cycloheximide, methotrexate and 2,4-dinitrophenol, and 30 observed much weaker transcript-response correlations when compared to ABA treatments (r=0.36, 0.73 and 0.81 respectively; cycloheximide shown in FIG. 1D). This demonstrates that an indirect developmental effect is not sufficient to account for the ABA-like transcriptional effects of pyrabac- 35

To establish if pyrabactin is a general ABA agonist, we examined its activity in seedlings treated with either compound for 24 hours, which showed that pyrabactin induces a greatly muted ABA response (r=0.72) in seedling tissues 40 (FIG. 1E). For seedling microarray experiments, Columbia wild type seeds were surface sterilized and sown on 0.5×MS, 0.6% (w/v) agar plates (15 mg seeds, 25 ml media per 150 mm plate), followed by stratification for 4 days at 4° C. and grown under 24-h light at room temperature for 9 days. 40 seedlings 45 were then transferred to either DMSO control, 10 µM ABA or 33 uM pyrabactin plates and returned to the growth environment for another 24 hours, after which total RNA was extracted using the method described above. Triplicate samples were hybridized per treatment. The concentrations 50 used for seedling experiments were based on concentrations of ABA or pyrabactin that are required to inhibit primary root growth by equivalent amounts, i.e. they were normalized to a measure of bioactivity. In these experiments, 57 transcripts responded significantly to both pyrabactin and ABA, suggesting that pyrabactin can induce aspects of an ABA response in seedlings However, since 3021 transcripts in this experiment showed a significant response to ABA, but not pyrabactin, we conclude that pyrabactin acts with greater selectivity for the seed pathway in comparison to ABA. Pyrabactin does agonize ABA responses in vegetative tissues.

PYR1, a START Protein, is Necessary for Pyrabactin Action To dissect pyrabactin's mechanism of action, we isolated a collection of 16 pyrabactin insensitive mutant lines from a screen of ~450,000 EMS mutagenized M2 seed. Surface sterilized EMS seeds were sown on 0.33×MS media containing 25 μM pyrabactin (50 mg seeds per 150 mm plate). Seeds

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were stratified for 4 days at 4° C. and grown under constant light for 4 days at room temperature, after which plates were scored for mutants resistant to the germination inhibition effect of pyrabactin. Seedlings with fully expanded cotyledons were considered resistant, and all mutants identified as resistant were then retested in the next generation to identify true mutants. The strong pyr1-7 allele was used to map Pyr1 using a mapping population of ~400 plants (created from progeny of a cross to Ler). This delimited Pyr1 to an ~150 Kb interval containing 12 genes. The identity of Pyr1 was first suggested after sequencing the 12 genes in this interval and identifying a stop codon in At4g17870 (Pyr1). After this, the Pyr1 coding sequence for 14 of the 16 mutations isolated were sequenced and 12 independent strains were determined by map based cloning and sequencing to contain mutations in the same locus, PYRABACTIN RESISTANCE 1 (Pyr1). Pyr1 encodes a protein that is a member of the START/Bet v 1 superfamily whose members share a conserved ligand-binding helix-grip architecture (L. M. Iyer, E. V. Koonin, L. Aravind, Proteins: Structure, Function, and Genetics 43, 134 (2001); C. Radauer, P. Lackner, H. Breiteneder, BMC Evol Biol 8, 286 (2008)). PYR1 resides in a Bet v 1 subfamily similar to bacterial polyketide synthases/cyclases and other non-enzymatic proteins (C. Radauer, P. Lackner, H. Breiteneder, BMC Evol Biol 8, 286 (2008)). There are 13 genes in the Arabidopsis genome that show significant similarity to Pyr1 in BLAST searches, which we have named PYL1-PYL13 (for PYR1-Like; their AGIs are listed in Table 1). The pyrabactin insensitive pyr1 alleles we isolated are predicted to produce a variety of defects in PYR1, including truncations and non-conservative amino acid substitutions (FIG. 2A). Transformation of a 35S::GFP-PYR1 expression construct into the strong pyr1-1 mutant line restores seed pyrabactin sensitivity (FIG. 2C), which provides further support that PYR1 is necessary for pyrabactin action. None of the pyr1 alleles isolated show strong ABA insensitivity, which as we describe below, is explained by the action of redundant Pyr1 relatives (including, but not limited to Pyl-1,2,4). By querying public microarray databases (M. Schmid et al., Nat Genet. 37, 501 (2005); K. Nakabayashi, M. Okamoto, T. Koshiba, Y. Kamiya, E. Nambara, Plant J41, 697 (March, 2005); H. Goda et al., *Plant J* 55, 526 (August, 2008); D. Winter et al., *PLoS* ONE 2, e718 (2007); Y. Yang, A. Costa, N. Leonhardt, R. S. Siegel, J. I. Schroeder, *Plant Methods* 4, 6 (2008)) it is clear that Pyr1 mRNA is expressed highly in seeds and guard cells and is responsive to ABA (FIG. 2B), consistent with a role for PYR1 in ABA signaling.

TABLE 1

		YL family and corresponding e Initative (AGI) annotations.	
	Gene	AGI	
5	Pyr1	AT4G17870	
,	Pyl1	AT5G46790	
	Pyl2	AT2G26040	
	Pyl3	AT1G73000	
	Pyl4	AT2G38310	
	Pyl5	AT5G05440	
0	Pyl6	AT2G40330	
0	Pyl7	AT4G01026	
	Pyl8	AT5G53160	
	Pyl9	AT1G01360	
	Pyl10	AT4G27920	
	Pyl11	AT5G45860	
	Pyl12	AT5G45870	
5	Pyl13	AT4G18620	

PYR/PYL Proteins Bind PP2Cs in Response to ABA

Given that PYR1 is necessary for pyrabactin action and is a predicted ligand-binding protein, we hypothesized that pyrabactin agonizes ABA signaling by inducing a proteinprotein interaction between PYR1 and a downstream effector. 5 To test this, ~2 million prey cDNA clones were screened against a PYR1 Y2H bait construct on media containing 10 μM pyrabactin. To create the PYR1 Y2H bait construct, the Pyr1 open reading frame was PCR amplified from genomic DNA and cloned to pGem-T easy vector (Promega). After sequence confirmation, the Pyr1 ORF was then cloned inframe between EcoRI and SalI sites of the pBD-GAL4 Cam vector (Stratagene) and transformed into yeast strain Y190. For the screen, an etiolated seedling cDNA library (J. Kim, K., Harter, A., Theologis, Proc Natl Acad Sci USA 94, 11786 (Oct. 28, 1997)) (ABRC stock CD4-22) was used. The cDNA library was first converted from phage to plasmid DNA, yielding 7.6×10<sup>7</sup> transformants. Plasmid DNA prepared from library was then used to transform Y190 as described in the GAL4 Two-Hybrid system manual (Stratagene). For each 20 screen, 40 µg of prey plasmid was transformed into 1 ml of competent Y190 cell harboring bait construct and then grown on SD agar plates lacking H is, Leu, and Trp, but containing 15 mM 3-AT and 10 μM pyrabactin. After 4 days incubation at 30° C., well-grown colonies were rescued and interactions 25 validated using filter lift assay or chloroform-agarose overlay method and X-Gal staining. This identified two pyrabactindependent hits which sequencing determined encoded cDNAs for the PP2C HAB1, a close relative of the wellcharacterized ABA response factor ABI1 (A. Saez et al., The 30 Plant Journal 37, 354 (2004); N. Leonhardt et al., THE PLANT CELL 16, 596 (2004)). Next, Y2H strains expressing an AD-HAB1 fusion protein and a BD-PYR1 fusion protein were grown on plates and tested for interactions in response to various compounds, all at 10 µM except for epi-brassinolide 35 (50 nM) and dimethyl sulfoxide (DMSO) (carrier solvent, 1%). When the pyrabactin-responsive PYR1-HAB1 Y2H strains were tested on (+)-ABA, strong interactions were observed by X-gal stain, but neither (-)-ABA, kinetin, 2,4-D, Gibberellic acid (GA), epi-brassinolide (BR), methyl jas- 40 monate (meJA) or apyrabactin showed activity (FIG. 3A). Thus, PYR1 interacts with HAB1 in a (+)-ABA dependent fashion.

To see if ABA and pyrabactin responsiveness is unique to PYR1, we tested 11 of the 13 PYL proteins as described 45 above, using Y2H strains expressing an AD-HAB1 fusion protein and a BD-PYR/PYL fusion protein (listed at the left of FIG. 3A). BD-PYR/PYL fusion proteins were constructed in the same manner as for BD-PYR1 above. This assay showed that PYL1-PYL4 interact with HAB1 in an ABA-stimulated 50 manner (FIG. 3A). Ligand-selective interactions are also observed for pyrabactin, which promotes interactions between HAB1 and PYR1, PYL1, or PYL3 (FIG. 3A). Of these, only Pyr1 is highly transcribed in seeds, which likely explains why mutations in Pyr1 cause the seeds to be insen- 55 sitive to pyrabactin. PYL2-PYL4 respond to both (+)-ABA and (-)-ABA (FIG. 3A), suggesting that they could be involved in both (+) and (-)-ABA responses. Notably, the remaining PYLs tested in the yeast two hybrid assay show constitutive interactions with HAB1, suggesting they may 60 have different thresholds for interaction with the PP2Cs from PYR1 and PYLs 1 to 4. However the interactions of PYLs 5-12 with the PP2Cs are indicative that the entire protein family is likely to share a similar mechanism of action involving PP2C modulation, as we describe below. Thus, we con- 65 clude that entire family modulates ABA responses via PP2C interactions.

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To investigate the ABA/pyrabactin responses further, we used the Y2H assay as described above to examine three substitution mutant proteins that cause strong pyrabactin insensitive phenotypes in plants. Two of the mutants tested, PYR1<sup>S152L</sup> and PYR1<sup>P88S</sup>, greatly reduce ABA induced PYR1-HAB1 interactions, while the PYR1<sup>R157H</sup> mutation does not affect the interaction (FIG. 3B). HAB1 possesses genetic redundancy with ABI1, ABI2 and other related PP2Cs (T. Yoshida et al., PLANT PHYSIOLOGY 140, 115 (2006)). We therefore tested ABI1 and ABI2 in the Y2H assay, using publicly available sequence validated cDNAs for ABI1 and ABI2 (C104649, and U24491 respectively). We observed that PYR1 interacts with wild type ABI1 and ABI2, but not the ABA insensitive protein  $ABI2^{G168D}$  encoded by abi2-1 (FIG. 3C). Thus, residues important to PYR1 and PP2C function in planta are important for the ABA response reconstituted in yeast. These in vivo interactions between PYR1 and PP2C likely occur in the cytoplasm and nucleoplasm, as suggested by the localization pattern observed for GFP-PYR1 (FIG. 4). PYR/PYL Proteins Act Redundantly in ABA Signaling

To examine whether the ABA-responsive PYL proteins act redundantly with PYR1 in ABA signaling, we isolated homozygous insertion alleles for PYL1, 2 and 4 from public insertion-allele collections (seed strains=Salk\_054640, GT\_2864, Sail\_517\_C08 respectively) (J. M. Alonso et al., Science 301, 653 (2003); A. Sessions et al., THE PLANT CELL 14, 2985 (2002); V. Sundaresan et al., Genes and Development 9, 1797 (1995)). The homozygous insertion lines and pyr1-1 were crossed to create pyr1-1:pyl2-1 and pyll-1:pyl4-1 heterozygous lines, which were then crossed to one another. ~70 progeny from this cross were genotyped by PCR to identify lines heterozygous for all 4 mutations, and 2 plants were identified. To assess if these lines segregated ABA insensitive plants, the F2 seed from a quadruple heterozygous plant were germinated on 0.7 µM (+)-ABA. Extensive variation in germination and growth was observed, and the most ABA-resistant seedlings were selected from ~1000 seed and genotyped by PCR and sequencing. None of the homozygous single mutant parents showed marked ABA insensitivity, but both a triple (pyr1-1, pyr1-1, pyl4-1) and quadruple (pyr1-1, pyr1-1, pyl2-1, pyl4-1) mutant line showed ABA insensitivity. The root and germination responses of the quadruple and triple mutants lines were examined in comparison to abi1-1, the strongest ABA-insensitive mutant isolated to date. For germination assays, seeds were stratified on plates containing (+)-ABA on 0.33×MS for 4 days at 4° C. and then germinated at 23° C. in the dark for 3 days at 90% RH. Seeds showing radicals 1/2 seed length or longer were scored as positive for germination. To investigate root growth, seeds were allowed to first germinate on MS plates after 4 days of stratification and then transferred to germinate at 23° C. in darkness at 90% RH. 48 hours post imbibition, seedlings showing radical emergence were transferred to (+)-ABA containing or control plates, grown vertically for 4 additional days in the dark and then new root growth measured. In germination assays, the quadruple mutant was more insensitive than the triple, but both exhibited a weaker phenotype than abi1-1 (FIG. 5A). In root growth assays, the quadruple and triple mutant lines both showed greater ABA insensitivity than abi1-1 (FIG. 5B). The quadruple mutant line also exhibits defects in ABA-induced gene expression. Quantitative RT-PCR experiments were conducted as described previously (H. Fujii, et al., *Plant Cell*, 19, 485 (2007)) using tagman probes identical to those described by Fujii et al. Briefly, 7 day old seedlings grown under continuous illumination on 0.3×MS plates were transferred to 0.3×MS media containing carrier solvent (0.1% DMSO) or 100 µM (+)-ABA for 5 hours, after which total

RNA was isolated using Qiagen plant RNeasy isolation kit. 5  $\mu g$  total RNA was used per 20  $\mu L$  first strand cDNA synthesis reaction using SuperScript Reverse Transcriptase. The reactions were diluted to  $100\,\mu l$  with TE and  $1.5\,\mu l$  of this was used in 15  $\mu L$  qRT-PCR reactions using taqman probes described 5 previously (6). Values shown are the average of triplicate measurements. Quadruple mutants exhibit decreased transcription of the ABA-responsive genes RD29 (FIG. 2D), NCED3 (FIG. 2E), and P5CS1 (FIG. 2E) in the presence of (+)-ABA. These experiments show that PYL1, PYL2 and 10 PYL4 function redundantly with PYR1 in the control of ABA-induced gene expression and germination and root responses to ABA.

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In Vitro Reconstitution of ABA Perception: ABA and PYR1 Inhibit PP2C Activity

To explore the functional implications of the PYR1-PP2C interaction, we examined if an ABA response could be reconstituted in vitro. Recombinant GST-HAB1, GST-ABI1 and GST-ABI2 were expressed in E. coli and tested for ligand-dependent interactions with 6×His-PYR1 in pull- 20 down assays. Purified 6×His-PYR1 and GST-HAB1 (20 and 100 μg respectively, 8 μM PYR1 final concentration), were combined in 100  $\mu l$  TBS containing 10  $\mu M$  (+)-ABA or 1% DMSO for negative control. The reaction was incubated for 90 minutes at RT and 5 µl of PrepEase (USB) His-tagged 25 protein purification resin was added. The resin and reaction mixture was incubated 30 min at RT with gentle shaking at 5 min intervals. The resin was washed five times with TBS containing 10 µM (+)-ABA. After the final wash, the bound protein was eluted in 20 µl SDS-PAGE buffer, boiled for 5 30 minutes and centrifuged. 5 µl of eluate was analyzed on SDS-PAGE. For pull-downs with ABI1 and ABI2, crude lysates were used in a similar method, except purified PP2C was replaced with cleared E. coli lysates. The amount of lysate added was determined by SDS-page analysis to yield 35 ~100 µg PP2C, such that the same stoichiometry was used as in assays using purified proteins. We found that both (+)-ABA and pyrabactin promote PP2C interactions with PYR1; however PYR1<sup>P888</sup> is insensitive in this assay (FIG. **6**A).

Since ABI1 and relatives are negative regulators of the 40 ABA signaling pathway, we hypothesized that the function of the ABA-promoted PYR1-PP2C interaction was to inhibit phosphatase activity and remove a negative input into the pathway, which would then promote signaling. To test this hypothesis, we examined the effects of (+)-ABA on PP2C 45 enzyme kinetics using recombinant GST-HAB1, 6×His-PYR1 or 6×His-PYR1<sup>P88S</sup> using the phosphatase substrate pNPP. The ORF of Arabidopsis HAB1 was amplified by PCR from a pUni clone obtained from the ABRC and cloned into pGex-2T to create a GST-HAB1 fusion protein. Both con- 50 structs were transformed into BL21[DE3]pLysS. For expression, cells harboring pGex-GST-HAB1 were grown overnight in 20 ml LB and then inoculated to 700 ml media containing 1 mM MnCl<sub>2</sub> and continued incubation with shaking at RT for 8 hr. Protein expression was then induced by 55 addition of IPTG to final concentration of 0.5 mM, and cells were cultured overnight at RT. Cells were then harvested by centrifugation at 4500 rpm for 20 min, resuspended in 10 ml TBS containing 10 mM MnCl<sub>2</sub>. Cells were stored at -80° C. To prepare cleared lysates, cells were freeze-thawed twice 60 and the lysate's viscosity reduced by shearing. The lysate was then spun at 12000×g for 10 min to yield the final cleared lysates. This was applied to 1 ml of immobilized glutathione column, washed with 20 ml of TBS and bound protein then eluted with 20 mM reduced glutathione. The eluate was dialyzed against TBS containing 10 mM MnCl<sub>2</sub>. MnCl<sub>2</sub> was used through purification steps and found to be critical for

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recovery of highly active HAB1 protein, as described previously for other PP2Cs (C. C. Field, J. M. Denu, J Biol Chem, 274, 20336 (Jul. 16, 1999)). The PYR1 and PYR1  $^{P88S}$  coding sequences were amplified by PCR from genomic DNA of wild type or the pyr1-3 mutant respectively and cloned into pET28 to produce various 6×His-PYR1 proteins, which were validated by sequencing. For 6×His-PYR1 and 6×His-PYR1<sup>P88S</sup> protein expressions, 20 ml of an overnight culture was inoculated to 700 ml LB and was grown for additional 3 hours at 37° C. with shaking. Protein expression was induced by addition of IPTG to 1 mM. Cells were harvested 5 hr later by centrifugation for 15 min at 5000×g and the pellet was resuspended in 5 ml of the Buffer A (50 mM NaH<sub>2</sub>PO<sub>4</sub>, 300 mM NaCl) containing 10 mM imidazole, pH 8.0). Cells were stored at -80° C. before purification. After thawing, cells were sonicated on ice five times for 30 sec with 30 sec resting intervals. A cleared lysate was obtained after centrifugation at 12,000×g for 10 min and applied to 1 ml of Ni-NTA column (Qiagen) and washed with 20 column volumes of Buffer A containing 30 mM imidazole. Bound protein was eluted with 10 ml of Buffer A with 100 mM imidazole. The eluate was dialyzed against TBS. For the pNPP assay, initial reaction velocities for GST-HAB1 were conducted using the synthetic phosphatase substrate pNPP. Reactions contained 1 µM GST-HAB1, 1.5 µM 6×His-PYR1 or 6×His-PYR1<sup>P88S</sup> and a reaction buffer consisting of 33 mM Tris-OAc, pH 7.9, 66 mM KOAc, 0.1% BSA, 25 mM Mg(OAc)<sub>2</sub>, 50 mM pNPP and varying (+)-ABA concentrations. Reactions were initiated by the addition of assay buffer to protein/ABA mixes. Immediately after mixing, reactions were monitored for hydrolysis of pNPP at A405 t ~10 second intervals over 20 minutes using a Wallac plate reader. Reaction progressions were plotted, initial velocities calculated and converted to specific activities using a standard curve for 4-nitrophenol made in the same buffer system volumes/plate reader used for enzymatic reaction measurements. These experiments show that (+)-ABA acts as a potent inhibitor of HAB1 phosphatase activity  $(IC_{50}=0.18 \mu M)$  in the presence of PYR1, but not PYR1<sup>P88S</sup> (FIG. 6B).

Similarly, ABA displays saturable inhibition of HAB1 PP2C activity in the presence of recombinant PYL4. A PYL4 6xHis-tagged (SEQ ID NO:141) protein was constructed using a public pUni clone. This was recombined into the His-tagged expression vector pHB3. The construct was expressed in BL21[DE3] pLysS as described above for PYR1, but the protein formed inclusion bodies, which were solubilized in Buffer B+8 M urea, prior to purification. The protein was purified under denaturing conditions using Ni-NTA resin according to manufacturer's instructions. After binding of protein to resin, the column was washed with 20 volume of Buffer B (pH6.3) and protein eluted using Buffer A (pH4.5). The eluted protein was dialyzed slowly from TBS containing 2 M urea, 10 mM DTT into TBS containing 1 mM DTT over three days, gradually lowering the urea concentration over time. The activity of refolded PYL4 was validated using in vitro pull down assays developed for PYR1, where it was shown that PYL4 binds HAB1 in response to ABA. For the PP2C assays, recombinant PYL4 (refolded from inclusion bodies) and HAB1 were used. When phosphatase activity was measured for GST-HAB1 using the phosphatase substrate pNPP, we found that (+)-ABA inhibits HAB1 phosphatase activity in the presence of PYL4 (FIG. 6C). Thus, PP2C inhibition is a primary ABA-response that can be reconstituted in vitro with only proteins.

5 Discussion

We have shown that PYR1 has the properties expected of an ABA receptor and that it binds to and inhibits PP2C activ-

ity when ligand is present. In contrast to previously identified ABA binding proteins (P. McCourt, R. Creelman, Current Opinion in Plant Biology 11, 474 (2008)), PYR1 interacts directly with core components of the ABA signaling pathway. ABI1 interacts with at least one positively acting factor in the 5 ABA response pathway (R. Yoshida et al., Journal of Biological Chemistry 281, 5310 (2006)). It may therefore be that the role of ABI1/AHG1 class PP2Cs in the absence of a signal is to repress the action of positively acting factors. In this model, ABA functions at the apex of a negative regulatory pathway 10 and the PP2Cs control signal output through their direct targets. This imbues the PP2Cs with a critical role in controlling the selectivity of signal-output, which could explain the extensive diversification of the PP2C gene family in plants relative to animals (A. Schweighofer, H. Hirt, I. Meskiene, 15 Trends in Plant Science 9, 236 (2004)). Based on the interaction of PP2Cs with SnRK2 proteins and the critical role of SnRK2s for ABA signaling (FIG. 7) we have proposed the following model for ABA action in which ABA and PYR/ PYLs inhibit the PP2Cs, which in turn relieves repression of 20 positive factors, such as the SnRK2s. This in turn allows the positive SnRK2 kinases to modulate activity of downstream factors via phosphorylation.

Our experiments show that at least 12 of the 14-members in the PYR/PYL gene family bind to PP2Cs, and some members 25 such as PYL2s, 3 and 4 enable yeast cells to respond to the unnatural stereoisomer (–)-ABA. We believe the entire family are ABA receptors and that some may also be (–)-ABA receptors. This hypothesis is consistent with earlier conclusions that both stereoisomers act through the same signaling 30 pathway (E. Nambara et al., *Genetics* 161, 1247 (July, 2002)).

PYR1 is unable to bind to the proteins encoded by abi1-1 and abi2-1, which both contain mutations in glycines near one of the two conserved PP2C metal binding sites. These mutations lower, but do not abolish, PP2C activity (F. Gosti et al., 35 The Plant Cell 11, 1897 (1999); N. Robert, S. Merlot, V. N'Guyen, A. Boisson-Dernier, J. I. Schroeder, FEBS Letters 580, 4691 (2006)) and a second site mutation that completely abolishes abi1-1's catalytic activity suppresses its dominant phenotype (F. Gosti et al., *The Plant Cell* 11, 1897 (1999)). 40 Together with our observations on defective PYR1 interactions, these data suggest a model where the dominance of the abi1-1 and abi2-1 mutations stems from their ability to escape negative regulation by the PYR/PYL proteins. In this model, a major function of ABA is to lower ABI1/AHG1 class PP2C 45 activity via PYR/PYL proteins, but this does not occur properly in the abi1-1 and abi2-1 mutant lines, which retain sufficient PP2C activity after ABA perception to disrupt signal

The regulation of PP2Cs is poorly understood with respect 50 to other phosphatase classes, which is surprising given their important roles in mammals, worms, flies and yeast (G. Lu, Y. Wang, Clinical and Experimental Pharmacology and Physiology 35, 107 (2008)). Our observations provide a new mechanism for receptor-mediated regulation of PP2C activ- 55 ity. Although the precise mechanism of PP2C inhibition by PYR1 is unknown, the PYR1<sup>R157H</sup> mutation is able to separate ligand perception from downstream functions in vivo. This residue may therefore play a critical role in steps that lead to inhibition of PP2C activity after signal perception. 60 Regardless of the precise details of PP2C inhibition, the novel regulatory mechanism discovered suggests that it may be worth investigating receptor-mediated PP2C regulation in other models, given the dearth of regulatory factors for these vital phosphatases.

The ABA signaling pathway has been the subject of genetic analysis for almost 30 years, but the PYR/PYL proteins never

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emerged as factors necessary for an ABA response in genetic screens. In hindsight, this is now obvious due to the necessity of a triple mutant to observe an ABA-insensitive phenotype. When using pyrabactin as a synthetic agonist of the pathway however, Pyr1 was identified with ease. The reason for this is due to pyrabactin's selectivity for a subset of the entire receptor family, which enabled us to bypass the genetic redundancy that obscures an ABA phenotype in single mutant lines. Thus, our results demonstrate the power of the chemical genetic approach to reveal phenotypes for normally redundant genes. Because plant genomes are highly redundant, we expect that small molecule approaches will provide a powerful addendum to classical genetic analysis.

## Example 2

# Screens for Agonists of PYR/PYL

We next investigated whether other compounds besides ABA and pyrabactin could act as agonists of PYR/PYL proteins. Yeast two hybrid strains expressing ABA-receptors and type 2 C protein phosphatases in the appropriate vectors can be used to monitor activation of ABA receptors. These yeast strains therefore create a facile screening system for the identification of cell permeant compounds that act as ABA agonists, i.e. compounds that promote binding of PYR/PYL family members to their protein phosphatase targets. When PYR/PYL proteins are bound to PP2C targets in the yeast two hybrid context, a reporter gene is activated which, depending on strains used, can lead to expression of a reporter construct such as the LacZ/B-galactoisidase marker or to a nutritional reporter gene that enables growth on auxotrophic media.

To conduct these agonist assays, screening compounds are added to microtiter wells and appropriate yeast growth media are added. The wells are then seeded with PYR/PYL-PP2C strains and agonist activity is monitored after growth of the strains on the chemical-containing medium. Numerous approaches can be used to monitor activation including simple growth (via restoration of expression of a nutritional reporter gene) of colorimetric X-gal assays, which are well known in the art. An alternative screening method, called the "Halo Assay," can also be used to identify agonists. In this assay, yeast strains can be embedded in suitable growth medium containing agarose and chemicals can be spotted onto plates using a pin replicator. The growth medium, lacking a nutrient needed for growth, prevents yeast growth unless one of the screening chemicals supplied enters the yeast cell and activates the PYR/PYL receptors, which results in expression of the nutritional marker genes in the yeast two hybrid strain. Activated cells appear as regions of cell growth and can be easily identified by visual inspection.

Using a combination of the conventional and halo assays as described above, 65,000 screening compounds were tested for activation of PYR1, PYL2, PYL3 or PYL4 expressing yeast two hybrid strains. Hit compounds that activated any of the yeast strains were retested on all 4 yeast strains and activity assessed qualitatively using X-gal staining assays. This led to the identification of the compounds shown in FIG. 8. Estimates of the relative activity of each of these compounds on the PYR/PYL receptors PYR1, PYL1, PYL2, PYL3, and PYL4 is depicted in FIG. 8. We note that the PYL3 yeast strain used in these screening assays is exceptionally sensitive to ABA, and therefore the estimate of the relative activity of ABA or other compounds on the PYL3 receptor may be refined by later performing in vitro phosphatase assays, described below.

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As a further validation of hit compounds identified in the

expressing the PYR/PYL receptor PYL4. We generated transgenic *Arabidopsis* plants expressing GFP-PYL4 under the control of the high expression promoter Rbcs, and found that plants that overexpress PYL4 exhibit defects in flowering time, stature, wiltiness, and the chlorophyll content of the

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plants that overexpress PYL4 exhibit defects in flowering time, stature, wiltiness, and the chlorophyll content of the plants; relative to control plants, these PYL4-overexpressing plants flower later, are darker green, and less wilty (FIG. 10). These results demonstrate that PYR/PYL receptors modulate a wide variety of ABA-mediated activities in plants.

# yeast two-hybrid assay, we utilized in vitro PP2C assays conducted in the presence of recombinant PYR/PYL receptor proteins PYR1, PYL1, PYL2, or PYL3 and the PP2C HAB1. Recombinant proteins were made as described above in 5 Example 1. Phosphatase assays using the phosphatase substrate pNPP were performed as described in Example 1. As demonstrated by the IC50 values, we found that compound 7653159, which is the same compound as compound 7 in FIG. 8, is a potent agonist of PYR1 and PYL1 inhibition of 10 HAB1 but is not an agonist for PYL2 or PYL3 (FIG. 9). Similarly, compound 6655097, which is the same compound as compound 6 in FIG. 8, is a potent agonist of PYR1 and PYL1 inhibition of HAB1 but is not an agonist for PYL2 or PYL3 (FIG. 9). Compound 7561035, which is the same com- 15 pound as compound 9 in FIG. 8, is a potent agonist of PYL2 and PYL3 inhibition of HAB1 but is not an agonist for PYR1 or PYL1 (FIG. 9).

#### Example 3

# Phenotypic Analysis of PYR/PYL Overexpression and Loss-of-Function Mutant Plants

Abscisic acid is a multifunctional phytohormone involved 25 in a variety of phyto-protective functions including bud dormancy, seed dormancy and/or maturation, abscission of leaves and fruits, and response to a wide variety of biological stresses (e.g. cold, heat, salinity, and drought). ABA is also responsible for regulating stomatal closure by a mechanism 30 independent of CO<sub>2</sub> concentration. Because PYR/PYL receptor proteins mediate ABA signaling, these phenotypes can be modulated by modulating expression of PYR/PYL. However, as discussed above, experiments with single, triple, and quadruple Pyr/Pyl mutant plants demonstrate that PYL receptors 35 PYL1, 2 and 4 function redundantly with PYR1 in the control of germination and root responses to ABA function. In these experiments, we asked whether other PYR/PYL receptors function redundantly with PYR1 in the control of plant phytoprotective functions such as flowering time, stature, chloro-40 phyll content, and wiltiness. We used the pyr1;pyl1;pyl2;pyl4 quadruple mutants as described above in Example 1 to test the effect of loss of function of multiple PYR/PYL receptors on these phyto-protective functions. We found that pyr1;pyl1; pyl2;pyl4 quadruple mutants exhibit defects in flowering 45 time, stature, and wiltiness (FIG. 10). Relative to a control Arabidopsis plant, pyr1;pyl1;pyl2;pyl4 quadruple mutants flower early, are smaller in stature, and are very wilty. We also examined the effect on phyto-protective functions from over-

# Example 4

## Screens of Plant Extracts for PYR/PYL Agonists

The yeast strains expressing PYR/PYL receptors and type 2 C protein phosphatases were also used to screen HPLCfractionated plant extracts for the presence of endogenous compounds that activate PYL/PYL receptors PYR1, PYL2, PYL3, and/or PYL4. HPLC fractionation of extracts was used 20 to identify compounds different from abscisic acid (the known agonist). This led to the identification of a PYL3/ PYL4 selective agonist in extracts made from Hypericum perforatum aerial tissues. Purification of the agonist was achieved via multiple rounds of chromatographic separation coupled to yeast two hybrid assays that informed the fractions to move forward at each step of the purification. The structure of the purified agonist was deduced by X-ray crystallography of crystalline purified agonist. This revealed the compound to be the previously known compound artemisinic acid. This compound has not been reported outside of the genus Artemisia (Asteraceae) and our isolation of this compound from Hypericum (Clusiaceae) suggests the compound may have widespread occurrence in plants, consistent with a functionally important role to plant physiology. Several related compounds were obtained from commercial sources and also found to possess PYL3/PYL4 selective agonist activity (FIG. 12). Following a similar approach to that described above for artemisinic acid, a second naturally occurring ABA agonist was identified from seeds of Cola accumulata and identified by 2D-NMR as a previously undescribed derivative of alphacopaene, copaenoic acid (FIG. 12).

It is understood that the examples and embodiments described herein are for illustrative purposes only and that various modifications or changes in light thereof will be suggested to persons skilled in the art and are to be included within the spirit and purview of this application and scope of the appended claims. All publications, patents, and patent applications cited herein are hereby incorporated by reference in their entirety for all purposes.

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Val 65	Trp	Ser	Val	Val	Arg 70	Arg	Phe	Asp	Lys	Pro 75	Gln	Thr	Tyr	Lys	His 80
Phe	Ile	Lys	Ser	Cys 85	His	Val	Glu	Asp	Gly 90	Phe	Glu	Met	Arg	Val 95	Gly
СЛа	Leu	Arg	Asp 100	Val	Asn	Val	Ile	Ser 105	Gly	Leu	Pro	Ala	Glu 110	Thr	Ser
Thr	Glu	Arg 115	Leu	Asp	Ile	Leu	Asp 120	Asp	Glu	Arg	His	Val 125	Thr	Gly	Phe
Ser	Ile 130	Ile	Gly	Gly	Glu	His 135	Arg	Leu	Arg	Asn	Tyr 140	Arg	Ser	Val	Thr
Thr 145	Val	His	Glu	Tyr	Gln 150	Asn	His	Gly	Gly	Glu 155	Ile	Trp	Thr	Val	Val 160
Leu	Glu	Ser	Tyr	Val 165	Val	Asp	Met	Pro	Glu 170	Gly	Asn	Thr	Glu	Glu 175	Asp
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< 400	) > SI	COUE	ICE:	6											
			NCE:		Ser	Ser	Thr	Ala	Ser	Thr	Ser	Asp	Gln	Asp	Ser
	O> SI Glu				Ser	Ser	Thr	Ala	Ser 10	Thr	Ser	Asp	Gln	Asp 15	Ser
Met 1		Lys	Ala	Glu 5					10					15	
Met 1 Asp	Glu	Lys Asn	Ala His 20	Glu 5 Arg	Thr	Gln	His	His 25	10 Leu	Thr	Leu	Pro	Ser 30	15 Gly	Leu
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Met 1 Asp Arg Thr Lys	Glu Gln Tyr 50 His	Lys Asn His 35 Leu Ala Gln	Ala His 20 Glu Ile Pro	Glu 5 Arg Phe Gly Pro Tyr 85	Thr Asp Pro Gln 70 Lys	Gln Ser Asn 55 Thr	His Leu 40 Gln Val	His 25 Ile Cys Trp	Leu Pro Ser Ser Lys 90	Thr Phe Thr Val 75 Ser	Leu Ile Leu 60 Val	Pro Asn 45 Leu Arg	Ser 30 Ser Ala Ser Leu	Gly His Gln Phe Lys 95	Leu His Arg Asp 80 Glu
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Met 1 Asp Arg Thr Lys Gly Glu	Glu Glu Gln Tyr 50 His Pro Phe Leu Arg	Lys Asn His 35 Leu Ala Gln Gln Pro 115 Arg	Ala His 20 Glu Ile Pro Ile Met 100 Ala Val	Glu 5 Arg Phe Gly Pro Tyr 85 Lys Ala Thr	Thr Asp Pro Gln 70 Lys Val Thr	Gln Ser Asn 55 Thr His Gly Ser Phe 135	His Leu 40 Gln Val Ile Cys Thr 120 Ser	His 25 Ile Cys Trp Ile Thr 105 Glu Ile	10 Leu Pro Ser Ser Lys 90 Arg	Thr Phe Thr Val 75 Ser Asp Leu Gly	Leu Ile Leu 60 Val Cys Val Asp Gly 140	Pro Asn 45 Leu Arg Ser Asn Val 125 Glu	Ser 30 Ser Ala Ser Leu Val 110 Leu	15 Gly His Gln Phe Lys 95 Ile Asp	Leu His Arg Asp 80 Glu Ser Asp
Met 1 Asp Arg Thr Ile 65 Lys Gly Gly Glu Lys 145	Glu Gln Tyr 50 His Pro Leu Arg 130	Lys Asn His 35 Leu Ala Gln Gln Pro 115 Arg	Ala His 20 Glu Ile Pro Ile Met 100 Ala Val	Glu 5 Arg Phe Gly Pro Tyr 85 Lys Ala Thr	Thr Asp Pro Gln 70 Lys Val Thr Gly Val 150	Gln Ser Asn 55 Thr His Gly Ser Phe 135	His Leu 40 Gln Val Ile Cys Thr 120 Ser	His 25 Ile Cys Trp Ile Thr 105 Glu Ile Val	10 Leu Pro Ser Ser Lys 90 Arg Tle	Thr Phe Thr Val 75 Ser Asp Leu Gly Gly 155	Leu Ile Leu 60 Val Cys Val Asp Gly 140 Phe	Pro Asn 45 Leu Arg Ser Asn Val 125 Glu Gly	Ser 30 Ser Ala Ser Leu Val 110 Leu His Asp	15 Gly His Gln Phe Lys 95 Ile Asp Arg Gly	Leu His Arg Asp 80 Glu Ser Asp Leu Asp
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Pro Ala Ala Glu Arg Ala Ala Gly Pro Gly Arg Arg Pro Thr Cys Thr Ser Leu Val Ala Gln Arg Val Asp Ala Pro Leu Ala Ala Val Trp Pro Ile Val Arg Gly Phe Ala Asn Pro Gln Arg Tyr Lys His Phe Ile Lys Ser Cys Glu Leu Ala Ala Gly Asp Gly Ala Thr Val Gly Ser Val Arg Glu Val Ala Val Val Ser Gly Leu Pro Ala Ser Thr Ser Thr Glu Arg Leu Glu Ile Leu Asp Asp Asp Arg His Val Leu Ser Phe Arg Val Val Gly Gly Asp His Arg Leu Arg Asn Tyr Arg Ser Val Thr Ser Val Thr 135 Glu Phe Ser Ser Pro Ser Ser Pro Pro Arg Pro Tyr Cys Val Val 150 155 Glu Ser Tyr Val Val Asp Val Pro Glu Gly Asn Thr Glu Glu Asp Thr Arg Met Phe Thr Asp Thr Val Val Lys Leu Asn Leu Gln Lys Leu Ala 185 Ala Val Ala Thr Ser Ser Ser Pro Pro Ala Ala Gly Asn His His 200 <210> SEQ ID NO 12 <211> LENGTH: 210 <212> TYPE: PRT <213> ORGANISM: Oryza sativa <220> FEATURE: <223> OTHER INFORMATION: rice Indica cultivar group, cultivar 93-11 hypothetical protein OsI\_06433 <400> SEQUENCE: 12 Met Glu Pro His Met Glu Arg Ala Leu Arg Glu Ala Val Ala Ser Glu Ala Glu Arg Arg Glu Leu Glu Gly Val Val Arg Ala His His Thr Phe Pro Ala Ala Glu Arg Ala Ala Gly Pro Gly Arg Arg Pro Thr Cys Thr Ser Leu Val Ala Gln Arg Val Asp Ala Pro Leu Ala Ala Val Trp Pro Ile Val Arg Gly Phe Ala Asn Pro Gln Arg Tyr Lys His Phe Ile Lys 65 70 75 80 Ser Cys Glu Leu Ala Ala Gly Asp Gly Ala Thr Val Gly Ser Val Arg Glu Val Ala Val Val Ser Gly Leu Pro Ala Ser Thr Ser Thr Glu Arg 105 Leu Glu Ile Leu Asp Asp Asp Arg His Val Leu Ser Phe Arg Val Val 120 Gly Gly Asp His Arg Leu Arg Asn Tyr Arg Ser Val Thr Ser Val Thr Glu Phe Ser Ser Pro Ser Ser Pro Pro Ser Pro Pro Arg Pro Tyr Cys Val Val Val Glu Ser Tyr Val Val Asp Val Pro Glu Gly Asn Thr Glu

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Сув	His	Val	Val 100	Val	Gly	Asp	Gly	Asp 105	Val	Gly	Thr	Leu	Arg 110	Glu	Val
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Val	Arg	Сув 195	Asn	Leu	Gln	Ser	Leu 200	Ala	Gln	Ile	Ala	Glu 205	Asn	Ala	Ala
Gly	Cys 210	Lys	Arg	Ser	Ser	Ser 215									
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Pro Pro Gly Asn Thr Arg Asp Glu Thr Cys Val Phe Val Asp Thr Ile 180 185 Val Lys Cys Asn Leu Thr Ser Leu Ser Gln Ile Ala Val Asn Val Asn 200 Arg Arg Lys Asp Ser 210 <210> SEQ ID NO 16 <211> LENGTH: 208 <212> TYPE: PRT <213> ORGANISM: Oryza sativa <220> FEATURE: <223> OTHER INFORMATION: rice Indica cultivar group, cultivar 93-11 hypothetical protein OsI\_04285 <400> SEQUENCE: 16 Met Pro Tyr Ala Ala Val Arg Pro Ser Pro Pro Pro Gln Leu Ser Arg Pro Ile Gly Ser Gly Ala Gly Gly Gly Lys Ala Cys Pro Ala Val Pro Cys Glu Val Ala Arg Tyr His Glu His Ala Val Gly Ala Gly Gln Cys 40 Cys Ser Thr Val Val Gln Ala Ile Ala Ala Pro Ala Asp Ala Val Trp Ser Val Val Arg Arg Phe Asp Arg Pro Gln Ala Tyr Lys Lys Phe Ile Lys Ser Cys Arg Leu Val Asp Gly Asp Gly Gly Glu Val Gly Ser Val Arg Glu Val Arg Val Val Ser Gly Leu Pro Ala Thr Ser Ser Arg Glu 105 Arg Leu Glu Val Leu Asp Asp Asp Arg Arg Val Leu Ser Phe Arg Ile 120 Val Gly Glu His Arg Leu Ala Asn Tyr Arg Ser Val Thr Thr Val His Glu Ala Ala Pro Ala Met Ala Val Val Glu Ser Tyr Val 150 155 Val Asp Val Pro Pro Gly Asn Thr Trp Glu Glu Thr Arg Val Phe Val Asp Thr Ile Val Arg Cys Asn Leu Gln Ser Leu Ala Arg Thr Val Glu 185 Arg Leu Ala Pro Glu Ala Pro Arg Ala Asn Gly Ser Ile Asp His Ala <210> SEQ ID NO 17 <211> LENGTH: 208 <212> TYPE: PRT <213> ORGANISM: Oryza sativa <220> FEATURE: <223> OTHER INFORMATION: rice Japonica cultivar group, cultivar Nipponbare, clone B1088C09 Bet v I allergen-like protein <400> SEQUENCE: 17 Met Pro Tyr Ala Ala Val Arg Pro Ser Pro Pro Pro Gln Leu Ser Arg 10 5 Pro Ile Gly Ser Gly Ala Gly Gly Lys Ala Cys Pro Ala Val Pro 25 Cys Glu Val Ala Arg Tyr His Glu His Ala Val Gly Ala Gly Gln Cys 40

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Phe Ser Thr Val Val Gln Ala Ile Ala Ala Pro Ala Asp Ala Val Trp Ser Val Val Arg Arg Phe Asp Arg Pro Gln Ala Tyr Lys Lys Phe Ile Lys Ser Cys Arg Leu Val Asp Gly Asp Gly Gly Glu Val Gly Ser Val Arg Glu Val Arg Val Val Ser Gly Leu Pro Ala Thr Ser Ser Arg Glu Arg Leu Glu Val Leu Asp Asp Asp Arg Arg Val Leu Ser Phe Arg Ile Val Gly Glu His Arg Leu Ala Asn Tyr Arg Ser Val Thr Thr Val His Glu Ala Ala Ala Pro Ala Met Ala Val Val Glu Ser Tyr Val Val Asp Val Pro Pro Gly Asn Thr Trp Glu Glu Thr Arg Val Phe Val Asp Thr Ile Val Arg Cys Asn Leu Gln Ser Leu Ala Arg Thr Val Glu Arg Leu Ala Pro Glu Ala Pro Arg Ala Asn Gly Ser Ile Asp His Ala <210> SEQ ID NO 18 <211> LENGTH: 213 <212> TYPE: PRT <213> ORGANISM: Picea sitchensis <220> FEATURE: <223> OTHER INFORMATION: Sitka spruce cultivar FB3-425, clone WS0276\_P02 unknown protein <400> SEQUENCE: 18 Met Asp Ile Ile Ala Gly Phe Asp Gln Leu Ser Phe Arg Leu Ser Gly Ala Ser Lys Gln Ile Thr Lys Thr Gly Ala Val Gln Tyr Leu Lys Gly Glu Glu Gly Tyr Gly Glu Trp Leu Lys Glu Val Met Gly Arg Tyr His 40 Tyr His Ser His Asp Gly Ala Arg Glu Cys Arg Cys Ser Ser Val Val Val Gln Gln Val Glu Ala Pro Val Ser Val Val Trp Ser Leu Val Arg Arg Phe Asp Gln Pro Gln Val Tyr Lys His Phe Val Ser Asn Cys Phe Met Arg Gly Asp Leu Lys Val Gly Cys Leu Arg Glu Val Arg Val Val Ser Gly Leu Pro Ala Ala Thr Ser Thr Glu Arg Leu Asp Ile Leu Asp 120 Glu Glu Arg His Ile Leu Ser Phe Ser Ile Val Gly Gly Asp His Arg 135 Leu Asn Asn Tyr Arg Ser Ile Thr Thr Leu His Glu Thr Leu Ile Asn 150 155 Gly Lys Pro Gly Thr Ile Val Ile Glu Ser Tyr Val Leu Asp Val Pro His Gly Asn Thr Lys Glu Glu Thr Cys Leu Phe Val Asp Thr Ile Val 185 Lys Cys Asn Leu Gln Ser Leu Ala His Val Ser Asn His Leu Asn Ser 200

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Thr His Arg Cys Leu
   210
<210> SEQ ID NO 19
<211> LENGTH: 207
<212> TYPE: PRT
<213> ORGANISM: Oryza sativa
<220> FEATURE:
<223> OTHER INFORMATION: rice Japonica cultivar group, cultivar
     Nipponbare (GA3) hypothetical protein Os06g0562200, Bet v I
     allergen family protein
<400> SEQUENCE: 19
Met Glu Ala His Val Glu Arg Ala Leu Arg Glu Gly Leu Thr Glu Glu
Glu Arg Ala Ala Leu Glu Pro Ala Val Met Ala His His Thr Phe Pro
Pro Ser Thr Thr Thr Ala Thr Thr Ala Ala Ala Thr Cys Thr Ser Leu
           40
Val Thr Gln Arg Val Ala Ala Pro Val Arg Ala Val Trp Pro Ile Val
                    55
Arg Ser Phe Gly Asn Pro Gln Arg Tyr Lys His Phe Val Arg Thr Cys
Ala Leu Ala Ala Gly Asp Gly Ala Ser Val Gly Ser Val Arg Glu Val
Thr Val Val Ser Gly Leu Pro Ala Ser Thr Ser Thr Glu Arg Leu Glu
                     105
Met Leu Asp Asp Asp Arg His Ile Ile Ser Phe Arg Val Val Gly Gly
                       120
Gln His Arg Leu Arg Asn Tyr Arg Ser Val Thr Ser Val Thr Glu Phe
Gln Pro Pro Ala Ala Gly Pro Gly Pro Ala Pro Pro Tyr Cys Val Val
           150
                           155
Val Glu Ser Tyr Val Val Asp Val Pro Asp Gly Asn Thr Ala Glu Asp
Thr Arg Met Phe Thr Asp Thr Val Val Lys Leu Asn Leu Gln Met Leu
                              185
Ala Ala Val Ala Glu Asp Ser Ser Ser Ala Ser Arg Arg Asp
<210> SEQ ID NO 20
<211> LENGTH: 216
<212> TYPE: PRT
<213> ORGANISM: Oryza sativa
<220> FEATURE:
<223> OTHER INFORMATION: rice Japonica cultivar group, cultivar
     Nipponbare (GA3) hypothetical protein Os05g0473000, Bet v I
     allergen family protein
<400> SEQUENCE: 20
Met Pro Tyr Thr Ala Pro Arg Pro Ser Pro Pro Gln His Ser Arg Ile
Gly Gly Cys Gly Gly Gly Val Leu Lys Ala Ala Gly Ala Ala Gly
                            25
His Ala Ala Ser Cys Val Ala Val Pro Ala Glu Val Ala Arg His His
              40
Glu His Ala Ala Gly Val Gly Gln Cys Cys Ser Ala Val Val Gln Ala
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Ile Ala Ala Pro Val Asp Ala Val Trp Ser Val Val Arg Arg Phe Asp Arg Pro Gln Ala Tyr Lys His Phe Ile Arg Ser Cys Arg Leu Leu Asp Gly Asp Gly Asp Gly Gly Ala Val Ala Val Gly Ser Val Arg Glu Val 105 Arg Val Val Ser Gly Leu Pro Ala Thr Ser Ser Arg Glu Arg Leu Glu Ile Leu Asp Asp Glu Arg Arg Val Leu Ser Phe Arg Val Val Gly Gly Glu His Arg Leu Ser Asn Tyr Arg Ser Val Thr Thr Val His Glu Thr Ala Ala Gly Ala Ala Ala Val Val Val Glu Ser Tyr Val Val Asp Val Pro His Gly Asn Thr Ala Asp Glu Thr Arg Met Phe Val Asp Thr Ile Val Arg Cys Asn Leu Gln Ser Leu Ala Arg Thr Ala Glu Gln Leu Ala Leu Ala Ala Pro Arg Ala Ala <210> SEO ID NO 21 <211> LENGTH: 212 <212> TYPE: PRT <213> ORGANISM: Vitis vinifera <220> FEATURE: <223> OTHER INFORMATION: grapevine cultivar PN40024 unnamed protein product, locus GSVIVT00029635001 <400> SEQUENCE: 21 Met Pro Ser Ser Leu Gln Leu His Arg Ile Asn Asn Ile Asp Pro Thr 10 Thr Val Ala Val Ala Ala Thr Ala Ala Val Asn Cys His Lys Gln Ser Arg Thr Pro Leu Arg Cys Ala Thr Pro Val Pro Asp Ala Val Ala Ser Tyr His Ala His Ala Val Gly Pro His Gln Cys Cys Ser Met Val Val Gln Thr Thr Ala Ala Ala Leu Pro Thr Val Trp Ser Val Val Arg Arg Phe Asp Asn Pro Gln Ala Tyr Lys His Phe Leu Lys Ser Cys His Val Ile Phe Gly Asp Gly Asp Ile Gly Thr Leu Arg Glu Val His Val Val Ser Gly Leu Pro Ala Glu Ser Ser Thr Glu Arg Leu Glu Ile Leu Asp 120 Asp Glu Arg His Val Leu Ser Phe Ser Val Val Gly Gly Asp His Arg Leu Cys Asn Tyr Arg Ser Val Thr Thr Leu His Pro Ser Pro Thr Gly 150 155 Thr Gly Thr Val Val Val Glu Ser Tyr Val Val Asp Ile Pro Pro Gly 170 Asn Thr Lys Glu Asp Thr Cys Val Phe Val Asp Thr Ile Val Lys Cys 185 Asn Leu Gln Ser Leu Ala Gln Met Ser Glu Lys Leu Thr Asn Asn Asn 200

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Arg Asn Ser Ser
   210
<210> SEQ ID NO 22
<211> LENGTH: 218
<212> TYPE: PRT
<213> ORGANISM: Zea mays
<220> FEATURE:
<223> OTHER INFORMATION: corn (maize) clone 1678999 cyclase/dehydrase
     family protein
<400> SEQUENCE: 22
Met Pro Cys Leu Gln Ala Ser Ser Pro Gly Ser Met Pro Tyr Gln His
His Gly Arg Gly Val Gly Cys Ala Ala Glu Ala Gly Ala Ala Val Gly
Ala Ser Ala Gly Thr Gly Thr Arg Cys Gly Ala His Asp Gly Glu Val
Pro Ala Glu Ala Ala Arg His His Glu His Ala Ala Pro Gly Pro Gly
Arg Cys Cys Ser Ala Val Val Gln Arg Val Ala Ala Pro Ala Glu Ala
Val Trp Ser Val Val Arg Arg Phe Asp Gln Pro Gln Ala Tyr Lys Arg
              85
                                 90
Phe Val Arg Ser Cys Ala Leu Leu Ala Gly Asp Gly Gly Val Gly Thr
           100
                             105
Leu Arg Glu Val Arg Val Val Ser Gly Leu Pro Ala Ala Ser Ser Arg
                          120
Glu Arg Leu Glu Val Leu Asp Asp Glu Ser His Val Leu Ser Phe Arg
                      135
Val Val Gly Gly Glu His Arg Leu Gln Asn Tyr Leu Ser Val Thr Thr
                 150
                                     155
Val His Pro Ser Pro Ala Ala Pro Asp Ala Ala Thr Val Val Val Glu
               165
                                   170
Ser Tyr Val Val Asp Val Pro Pro Gly Asn Thr Pro Glu Asp Thr Arg
Val Phe Val Asp Thr Ile Val Lys Cys Asn Leu Gln Ser Leu Ala Thr
              200
Thr Ala Glu Lys Leu Ala Leu Ala Ala Val
<210> SEQ ID NO 23
<211> LENGTH: 179
<212> TYPE: PRT
<213 > ORGANISM: Physcomitrella patens
<220> FEATURE:
<223> OTHER INFORMATION: moss Physcomitrella patens subspecies patens,
      ecotype Gransden 2004 predicted protein, locus
     PHYPADRAFT_222359
<400> SEQUENCE: 23
Met Gln Thr Lys Gly Arg Gln Ala Asp Phe Gln Thr Leu Leu Glu Gly
                                  1.0
Gln Gln Asp Leu Ile Cys Arg Phe His Arg His Glu Leu Gln Pro His
Gln Cys Gly Ser Ile Leu Leu Gln Leu Ile Lys Ala Pro Val Glu Thr
Val Trp Ser Val Ala Arg Ser Phe Asp Lys Pro Gln Val Tyr Lys Arg
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Phe Ile Gln Thr Cys Glu Ile Ile Glu Gly Asp Gly Gly Val Gly Ser 70 75 Ile Arg Glu Val Arg Leu Val Ser Ser Ile Pro Ala Thr Ser Ser Ile Glu Arg Leu Glu Ile Leu Asp Asp Glu Glu His Ile Ile Ser Phe Arg Val Leu Gly Gly Gly His Arg Leu Gln Asn Tyr Trp Ser Val Thr Ser Leu His Ser His Glu Ile Asp Gly Gln Met Gly Thr Leu Val Leu Glu Ser Tyr Val Val Asp Ile Pro Glu Gly Asn Thr Arg Glu Glu Thr His Met Phe Val Asp Thr Val Val Arg Cys Asn Leu Lys Ala Leu Ala Gln Val Ser Glu <210> SEQ ID NO 24 <211> LENGTH: 229 <212> TYPE: PRT <213> ORGANISM: Oryza sativa <220> FEATURE: <223> OTHER INFORMATION: rice Indica cultivar group, cultivar 93-11 hypothetical protein OsI\_11160 <400> SEOUENCE: 24 Met Pro Cys Ile Pro Ala Ser Ser Pro Gly Ile Pro His Gln His Gln 10 His Gln His His Arg Ala Leu Ala Gly Val Gly Met Ala Val Gly Cys 25 Ala Ala Glu Ala Ala Val Ala Ala Ala Gly Val Ala Gly Thr Arg Cys 40 Gly Ala His Asp Gly Glu Val Pro Met Glu Val Ala Arg His His Glu His Ala Glu Pro Gly Ser Gly Arg Cys Cys Ser Ala Val Val Gln His Val Ala Ala Pro Ala Pro Ala Val Trp Ser Val Val Arg Arg Phe Asp Gln Pro Gln Ala Tyr Lys Arg Phe Val Arg Ser Cys Ala Leu Leu Ala Gly Asp Gly Gly Val Gly Thr Leu Arg Glu Val Arg Val Val Ser Gly Leu Pro Ala Ala Ser Ser Arg Glu Arg Leu Glu Ile Leu Asp Asp Glu Ser His Val Leu Ser Phe Arg Val Val Gly Gly Glu His Arg Leu Lys Asn Tyr Leu Ser Val Thr Thr Val His Pro Ser Pro Ser Ala Pro Thr 170 Ala Ala Thr Val Val Val Glu Ser Tyr Val Val Asp Val Pro Pro Gly 185 Asn Thr Pro Glu Asp Thr Arg Val Phe Val Asp Thr Ile Val Lys Cys Asn Leu Gln Ser Leu Ala Lys Thr Ala Glu Lys Leu Ala Ala Gly Ala 215 220

Arg Ala Ala Gly Ser

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225
<210> SEQ ID NO 25
<211> LENGTH: 229
<212> TYPE: PRT
<213> ORGANISM: Oryza sativa
<220> FEATURE:
<223> OTHER INFORMATION: rice Japonica cultivar group, cultivar
     Nipponbare (GA3) hypothetical protein Os03g0297600, Bet v I
     allergen family protein
<400> SEQUENCE: 25
Met Pro Cys Ile Pro Ala Ser Ser Pro Gly Ile Pro His Gln His Gln
His Gln His His Arg Ala Leu Ala Gly Val Gly Met Ala Val Gly Cys
Ala Ala Glu Ala Ala Val Ala Ala Ala Gly Val Ala Gly Thr Arg Cys
Gly Ala His Asp Gly Glu Val Pro Met Glu Val Ala Arg His His Glu
His Ala Glu Pro Gly Ser Gly Arg Cys Cys Ser Ala Val Val Gln His
Val Ala Ala Pro Ala Ala Ala Val Trp Ser Val Val Arg Arg Phe Asp
Gln Pro Gln Ala Tyr Lys Arg Phe Val Arg Ser Cys Ala Leu Leu Ala
Gly Asp Gly Gly Val Gly Thr Leu Arg Glu Val Arg Val Val Ser Gly
                 120
Leu Pro Ala Ala Ser Ser Arg Glu Arg Leu Glu Ile Leu Asp Asp Glu
                 135
Ser His Val Leu Ser Phe Arg Val Val Gly Gly Glu His Arg Leu Lys
                 150
                                     155
Asn Tyr Leu Ser Val Thr Thr Val His Pro Ser Pro Ser Ala Pro Thr
Ala Ala Thr Val Val Val Glu Ser Tyr Val Val Asp Val Pro Pro Gly
                   185
Asn Thr Pro Glu Asp Thr Arg Val Phe Val Asp Thr Ile Val Lys Cys
                        200
Asn Leu Gln Ser Leu Ala Lys Thr Ala Glu Lys Leu Ala Ala Gly Ala
Arg Ala Ala Gly Ser
<210> SEQ ID NO 26
<211> LENGTH: 205
<212> TYPE: PRT
<213 > ORGANISM: Medicago truncatula
<220> FEATURE:
<223> OTHER INFORMATION: barrel medic unkown protein, clone
     MTYFP_FQ_FR_FS1G-H-19
<400> SEQUENCE: 26
Met Pro Ser Pro Val Gln Phe Gln Arg Phe Asp Ser Asn Thr Ala Ile
1 5
                     10 15
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Thr Asn Gly Val Asn Cys Pro Lys Gln Ile Gln Ala Cys Arg Tyr Ala 25

Leu Ser Ser Leu Lys Pro Thr Val Ser Val Pro Glu Thr Val Val Asp 40

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His His Met His Val Val Gly Gln Asn Gln Cys Tyr Ser Val Val Ile Gln Thr Ile Asn Ala Ser Val Ser Thr Val Trp Ser Val Val Arg Arg Phe Asp Tyr Pro Gln Gly Tyr Lys His Phe Val Lys Ser Cys Asn Val Val Ala Ser Gly Asp Gly Ile Arg Val Gly Ala Leu Arg Glu Val Arg Leu Val Ser Gly Leu Pro Ala Val Ser Ser Thr Glu Arg Leu Asp Ile Leu Asp Glu Glu Arg His Val Ile Ser Phe Ser Val Val Gly Gly Val His Arg Cys Arg Asn Tyr Arg Ser Val Thr Thr Leu His Gly Asp Gly Asn Gly Gly Thr Val Val Ile Glu Ser Tyr Val Val Asp Val Pro Gln Gly Asn Thr Lys Glu Glu Thr Cys Ser Phe Ala Asp Thr Ile Val Arg Cys Asn Leu Gln Ser Leu Val Gln Ile Ala Glu Lys Leu <210> SEQ ID NO 27 <211> LENGTH: 212 <212> TYPE: PRT <213> ORGANISM: Zea mays <220> FEATURE: <223> OTHER INFORMATION: corn (maize) clone 1458362 AT-rich element binding factor 3 <400> SEQUENCE: 27 Met Pro Phe Ala Ala Ser Arg Thr Ser Gln Gln Gln His Ser Arg Val Ala Thr Asn Gly Arg Ala Val Ala Val Cys Ala Gly His Ala Gly Val Pro Asp Glu Val Ala Arg His His Glu His Ala Val Ala Ala Gly Gln Cys Cys Ala Ala Met Val Gln Ser Ile Ala Ala Pro Val Asp Ala Val Trp Ser Leu Val Arg Arg Phe Asp Gln Pro Gln Arg Tyr Lys Arg Phe Ile Arg Ser Cys His Leu Val Asp Gly Asp Gly Ala Glu Val Gly Ser Val Arg Glu Leu Leu Val Ser Gly Leu Pro Ala Glu Ser Ser Arg Glu Arg Leu Glu Ile Arg Asp Asp Glu Arg Arg Val Ile Ser Phe Arg Val Leu Gly Gly Asp His Arg Leu Ala Asn Tyr Arg Ser Val Thr Thr Val His Glu Ala Ala Pro Ser Gln Asp Gly Arg Pro Leu Thr Met Val 155 Val Glu Ser Tyr Val Val Asp Val Pro Pro Gly Asn Thr Val Glu Glu Thr Arg Ile Phe Val Asp Thr Ile Val Arg Cys Asn Leu Gln Ser Leu 185 Glu Gly Thr Val Ile Arg Gln Leu Glu Ile Ala Ala Met Pro His Asp

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Asp Asn Gln Asn
   210
<210> SEQ ID NO 28
<211> LENGTH: 233
<212> TYPE: PRT
<213> ORGANISM: Zea mays
<220> FEATURE:
<223> OTHER INFORMATION: corn (maize) strain B73, clone ZM_BFb0105018
     unknown protein
<400> SEQUENCE: 28
Met Arg Glu Arg Asn Ser Ser Ile Asp Gln Glu His Gln Arg Gly Ser
Ser Ser Arg Ser Thr Met Pro Phe Ala Ala Ser Arg Thr Ser Gln Gln
Gln His Ser Arg Val Ala Thr Asn Gly Arg Ala Val Ala Val Cys Ala
Gly His Ala Gly Val Pro Asp Glu Val Ala Arg His His Glu His Ala 50 \, 55 \, 60 \,
Val Ala Ala Gly Gln Cys Cys Ala Ala Met Val Gln Ser Ile Ala Ala 65 70 75 80
Pro Val Asp Ala Val Trp Ser Leu Val Arg Arg Phe Asp Gln Pro Gln
                                  90
Arg Tyr Lys Arg Phe Ile Arg Ser Cys His Leu Val Asp Gly Asp Gly
                              105
Ala Glu Val Gly Ser Val Arg Glu Leu Leu Leu Val Ser Gly Leu Pro
Ala Glu Ser Ser Arg Glu Arg Leu Glu Ile Arg Asp Asp Glu Arg Arg
               135
Val Ile Ser Phe Arg Val Leu Gly Gly Asp His Arg Leu Ala Asn Tyr
        150
                                     155
Arg Ser Val Thr Thr Val His Glu Ala Ala Pro Ser Gln Asp Gly Arg
Pro Leu Thr Met Val Val Glu Ser Tyr Val Val Asp Val Pro Pro Gly
Asn Thr Val Glu Glu Thr Arg Ile Phe Val Asp Thr Ile Val Arg Cys
                200
Asn Leu Gln Ser Leu Glu Gly Thr Val Ile Arg Gln Leu Glu Ile Ala
Ala Met Pro His Asp Asp Asn Gln Asn
<210> SEQ ID NO 29
<211> LENGTH: 194
<212> TYPE: PRT
<213 > ORGANISM: Physcomitrella patens
<220> FEATURE:
<223> OTHER INFORMATION: moss Physcomitrella patens subspecies patens,
      ecotype Gransden 2004 predicted hypothetical
     protein, locus PHYPADRAFT_209242
<400> SEQUENCE: 29
Met Met Gln Glu Lys Gln Gly Arg Pro Asp Phe Gln Phe Leu Leu Glu
Gly Gln Gln Asp Leu Ile Cys Arg Phe His Lys His Glu Leu Leu Pro
His Gln Cys Gly Ser Ile Leu Leu Gln Gln Ile Lys Ala Pro Val Gln
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Thr Val Trp Leu Ile Val Arg Arg Phe Asp Glu Pro Gln Val Tyr Lys 55 Arg Phe Ile Gln Arg Cys Asp Ile Val Glu Gly Asp Gly Val Val Gly Ser Ile Arg Glu Val Gln Leu Val Ser Ser Ile Pro Ala Thr Ser Ser Ile Glu Arg Leu Glu Ile Leu Asp Asp Glu Glu His Ile Ile Ser Phe Arg Val Leu Gly Gly Gly His Arg Leu Gln Asn Tyr Trp Ser Val Thr Ser Leu His Arg His Glu Ile Gln Gly Gln Met Gly Thr Leu Val Leu Glu Ser Tyr Val Val Asp Ile Pro Asp Gly Asn Thr Arg Glu Glu Thr His Thr Phe Val Asp Thr Val Val Arg Cys Asn Leu Lys Ala Leu Ala Gln Val Ser Glu Gln Lys His Leu Leu Asn Ser Asn Glu Lys Pro Ala 185 Ala Pro <210> SEQ ID NO 30 <211> LENGTH: 191 <212> TYPE: PRT <213> ORGANISM: Vitis vinifera <220> FEATURE: <223> OTHER INFORMATION: grapevine cultivar PN40024 unnamed protein product, locus GSVIVT00035869001 <400> SEQUENCE: 30 Met Lys Val Tyr Ser Pro Ser Gln Ile Leu Ala Glu Arg Gly Pro Arg 10 Ala Gln Ala Met Gly Asn Leu Tyr His Thr His His Leu Leu Pro Asn 25 Gln Cys Ser Ser Leu Val Val Gln Thr Thr Asp Ala Pro Leu Pro Gln Val Trp Ser Met Val Arg Arg Phe Asp Arg Pro Gln Ser Tyr Lys Arg Phe Val Arg Gly Cys Thr Leu Arg Arg Gly Lys Gly Gly Val Gly Ser 65 70 75 80 Val Arg Glu Val Asn Ile Val Ser Gly Leu Pro Ala Glu Ile Ser Leu Glu Arg Leu Asp Lys Leu Asp Asp Leu His Val Met Arg Phe Thr Val Ile Gly Gly Asp His Arg Leu Ala Asn Tyr His Ser Thr Leu Thr Leu His Glu Asp Glu Glu Asp Gly Val Arg Lys Thr Val Val Met Glu 135 Ser Tyr Val Val Asp Val Pro Gly Gly Asn Ser Ala Gly Glu Thr Cys 150 155 Tyr Phe Ala Asn Thr Ile Ile Gly Phe Asn Leu Lys Ala Leu Ala Ala Val Thr Glu Thr Met Ala Leu Lys Ala Asn Ile Pro Ser Gly Phe 185

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<210> SEQ ID NO 31
<211> LENGTH: 217
<212> TYPE: PRT
<213> ORGANISM: Physcomitrella patens
<220> FEATURE:
<223> OTHER INFORMATION: moss Physcomitrella patens subspecies patens,
     ecotype Gransden 2004 predicted hypothetical
     protein, locus PHYPADRAFT_132509
<400> SEQUENCE: 31
Met Gln Gln Val Lys Gly Arg Gln Asp Phe Gln Arg Leu Leu Glu Ala
Gln Gln Asp Leu Ile Cys Arg Tyr His Thr His Glu Leu Lys Ala His
Gln Cys Gly Ser Ile Leu Leu Gln Gln Ile Lys Val Pro Leu Pro Ile
Val Trp Ala Ile Val Arg Ser Phe Asp Lys Pro Gln Val Tyr Lys Arg
Phe Ile Gln Thr Cys Lys Ile Thr Glu Gly Asp Gly Gly Val Gly Ser
Ile Arg Glu Val His Leu Val Ser Ser Val Pro Ala Thr Cys Ser Ile
Glu Arg Leu Glu Ile Leu Asp Asp Glu Lys His Ile Ile Ser Phe Arg
          100
                             105
Val Leu Gly Gly Gly His Arg Leu Gln Asn Tyr Ser Ser Val Ser Ser
Leu His Glu Leu Glu Val Glu Gly His Pro Cys Thr Leu Val Leu Glu
                      135
Ser Tyr Met Val Asp Ile Pro Asp Gly Asn Thr Arg Glu Glu Thr His
                 150
                            155
Met Phe Val Asp Thr Val Val Arg Cys Asn Leu Lys Ser Leu Ala Gln
              165
                                 170
Ile Ser Glu Gln Gln Tyr Asn Lys Asp Cys Leu Gln Gln Lys Gln His
Asp Gln Gln Met Tyr Gln Gln Arg His Pro Pro Leu Pro Pro Ile
                           200
Pro Ile Thr Asp Lys Asn Met Glu Arg
<210> SEQ ID NO 32
<211> LENGTH: 195
<212> TYPE: PRT
<213 > ORGANISM: Physcomitrella patens
<223 > OTHER INFORMATION: moss Physcomitrella patens subspecies patens,
     ecotype Gransden 2004 predicted hypothetical
     protein, locus PHYPADRAFT_213389
<400> SEQUENCE: 32
Met Arg Phe Asp Ile Gly His Asn Asp Val Arg Gly Phe Phe Thr Cys
                                 10
     5
Glu Glu Glu His Ala Tyr Ala Leu His Ser Gln Thr Val Glu Leu Asn
                     25
Gln Cys Gly Ser Ile Leu Met Gln Gln Ile His Ala Pro Ile Glu Val
                          40
Val Trp Ser Ile Val Arg Ser Phe Gly Ser Pro Gln Ile Tyr Lys Lys
                      55
Phe Ile Gln Ala Cys Ile Leu Thr Val Gly Asp Gly Gly Val Gly Ser
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Ile Arg Glu Val Phe Leu Val Ser Gly Val Pro Ala Thr Ser Ser Ile 90 Glu Arg Leu Glu Ile Leu Asp Asp Glu Lys His Val Phe Ser Phe Arg 105 Val Leu Lys Gly Gly His Arg Leu Gln Asn Tyr Arg Ser Val Thr Thr Leu His Glu Gln Glu Val Asn Gly Arg Gln Thr Thr Thr Val Leu Glu Ser Tyr Val Val Asp Val Pro Asp Gly Asn Thr Arg Glu Glu Thr His Met Phe Ala Asp Thr Val Val Met Cys Asn Leu Lys Ser Leu Ala Gln Val Ala Glu Trp Arg Ala Met Gln Gly Ile Thr Gln Gln Leu Ser Thr Ser Ser Leu 195 <210> SEQ ID NO 33 <211> LENGTH: 172 <212> TYPE: PRT <213> ORGANISM: Vitis vinifera <220> FEATURE: <223> OTHER INFORMATION: grapevine cultivar Pinot Noir, clone ENTAV 115 hypothetical protein, locus VITISV\_004947 <400> SEOUENCE: 33 Met Gly Asn Leu Tyr His Thr His His Leu Leu Pro Asn Gln Cys Ser Ser Leu Val Val Gln Thr Thr Asp Ala Pro Leu Pro Gln Val Trp Ser 25 Met Val Arg Arg Phe Asp Arg Pro Gln Ser Tyr Lys Arg Phe Val Arg Gly Cys Thr Leu Arg Arg Gly Lys Gly Gly Val Gly Ser Val Arg Glu Val Asn Ile Val Ser Gly Leu Pro Ala Glu Ile Ser Leu Glu Arg Leu Asp Lys Leu Asp Asp Asp Leu His Val Met Arg Phe Thr Val Ile Gly Gly Asp His Arg Leu Ala Asn Tyr His Ser Thr Leu Thr Leu His Glu Asp Glu Glu Asp Gly Val Arg Lys Thr Val Val Met Glu Ser Tyr Val Val Asp Val Pro Gly Gly Asn Ser Ala Gly Glu Thr Cys Tyr Phe Ala Asn Thr Ile Ile Gly Phe Asn Leu Lys Ala Leu Ala Ala Val Thr Glu Thr Met Ala Leu Lys Ala Asn Ile Pro Ser Gly Phe 165 <210> SEQ ID NO 34 <211> LENGTH: 196 <212> TYPE: PRT <213> ORGANISM: Picea sitchensis <220> FEATURE: <223> OTHER INFORMATION: Sitka spruce cultivar FB3-425, clone WS0281\_I24 unknown protein

<400> SEQUENCE: 34

Met Glu Asp Leu Ser Ser Trp Arg Glu Gly Arg Ala Met Trp Leu Gly Asn Pro Pro Ser Glu Ser Glu Leu Val Cys Arg His His Arg His Glu Leu Gln Gly Asn Gln Cys Ser Ser Phe Leu Val Lys His Ile Arg Ala Pro Val His Leu Val Trp Ser Ile Val Arg Thr Phe Asp Gln Pro Gln Lys Tyr Lys Pro Phe Val His Ser Cys Ser Val Arg Gly Gly Ile Thr Val Gly Ser Ile Arg Asn Val Asn Val Lys Ser Gly Leu Pro Ala Thr Ala Ser Glu Glu Arg Leu Glu Ile Leu Asp Asp Asn Glu His Val Phe Ser Ile Lys Ile Leu Gly Gly Asp His Arg Leu Gln Asn Tyr Ser Ser 120 Ile Ile Thr Val His Pro Glu Ile Ile Asp Gly Arg Pro Gly Thr Leu 135 Val Ile Glu Ser Tyr Val Val Asp Val Pro Glu Gly Asn Thr Arg Glu 150 Glu Thr Arg Phe Phe Val Glu Ala Leu Val Lys Cys Asn Leu Lys Ser Leu Ala Asp Val Ser Glu Arg Leu Ala Ser Gln His His Thr Glu Leu 185 Leu Glu Arg Thr 195 <210> SEQ ID NO 35 <211> LENGTH: 185 <212> TYPE: PRT <213> ORGANISM: Solanum tuberosum <220> FEATURE: <223> OTHER INFORMATION: potato cultivar Kuras, clone 153D02 CAPIP1-like protein, similar to Capsicum annuum antimicrobial protein (CAPIP1) <400> SEQUENCE: 35 Met Asn Ala Asn Gly Phe Cys Gly Val Glu Lys Glu Tyr Ile Arg Lys His His Leu His Glu Pro Lys Glu Asn Gln Cys Ser Ser Phe Leu Val Lys His Ile Arg Ala Pro Val His Leu Val Trp Ser Leu Val Arg Arg Phe Asp Gln Pro Gln Lys Tyr Lys Pro Phe Ile Ser Arg Cys Ile Val Gln Gly Asp Leu Glu Ile Gly Ser Leu Arg Glu Val Asp Val Lys Ser Gly Leu Pro Ala Thr Thr Ser Thr Glu Arg Leu Glu Leu Leu Asp Asp Glu Glu His Ile Leu Ser Val Arg Ile Val Gly Gly Asp His Arg Leu Arg Asn Tyr Ser Ser Val Ile Ser Val His Pro Glu Val Ile Asp Gly 120 Arg Pro Gly Thr Val Val Leu Glu Ser Phe Val Val Asp Val Pro Glu

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Gly Asn Thr Lys Asp Glu Thr Cys Tyr Phe Val Glu Ala Leu Ile Asn
145
                   150
                                       155
Cys Asn Leu Lys Ser Leu Ala Asp Ile Ser Glu Arg Val Ala Val Gln
Asp Arg Thr Glu Pro Ile Asp Gln Val
         180
<210> SEQ ID NO 36
<211> LENGTH: 190
<212> TYPE: PRT
<213> ORGANISM: Medicago truncatula
<223> OTHER INFORMATION: barrel medic unkown protein, clone
     MTYFP_FQ_FR_FS1G-E-17
<400> SEQUENCE: 36
Met Asn Asn Gly Cys Glu Gln Gln Gln Tyr Ser Val Ile Glu Thr Gln
Tyr Ile Arg Arg His His Lys His Asp Leu Arg Asp Asn Gln Cys Ser
Ser Ala Leu Val Lys His Ile Lys Ala Pro Val His Leu Val Trp Ser
Leu Val Arg Arg Phe Asp Gln Pro Gln Lys Tyr Lys Pro Phe Ile Ser
Arg Cys Ile Met Gln Gly Asp Leu Ser Ile Gly Ser Val Arg Glu Val 65 70 75 80
Asn Val Lys Ser Gly Leu Pro Ala Thr Thr Ser Thr Glu Arg Leu Glu
Gln Leu Asp Asp Glu Glu His Ile Leu Gly Ile Arg Ile Val Gly Gly
                              105
Asp His Arg Leu Arg Asn Tyr Ser Ser Ile Ile Thr Val His Pro Gly
                          120
Val Ile Asp Gly Arg Pro Gly Thr Met Val Ile Glu Ser Phe Val Val
Asp Val Pro Glu Gly Asn Thr Lys Asp Glu Thr Cys Tyr Phe Val Glu
                   150
                                       155
Ala Leu Ile Arg Tyr Asn Leu Ser Ser Leu Ala Asp Val Ser Glu Arg
Met Ala Val Gln Gly Arg Thr Asp Pro Ile Asn Ile Asn Pro
                               185
<210> SEQ ID NO 37
<211> LENGTH: 185
<212> TYPE: PRT
<213> ORGANISM: Vitis vinifera
<220> FEATURE:
<223> OTHER INFORMATION: grapevine cultivar PN40024 unnamed protein
     product, locus GSVIVT00002440001
<400> SEQUENCE: 37
Met Ser Gly Tyr Gly Cys Ile Lys Met Glu Asp Glu Tyr Ile Arg Arg
His His Arg His Glu Ile Arg Asp Asn Gln Cys Ser Ser Leu Val
                              25
Lys His Ile Lys Ala Pro Val His Leu Val Trp Ser Leu Val Arg Ser
                40
Phe Asp Gln Pro Gln Lys Tyr Lys Pro Phe Val Ser Arg Cys Ile Val
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Gln Gly Asp Leu Glu Ile Gly Ser Val Arg Glu Val Asn Val Lys Ser
           70
                                       75
Gly Leu Pro Ala Thr Thr Ser Thr Glu Arg Leu Glu Leu Leu Asp Asp
Glu Glu His Ile Phe Gly Met Arg Ile Val Gly Gly Asp His Arg Leu
                 105
Lys Asn Tyr Ser Ser Ile Val Thr Val His Pro Glu Ile Ile Asp Gly
Arg Pro Gly Thr Leu Val Ile Glu Ser Phe Val Val Asp Val Pro Asp
Gly Asn Thr Lys Asp Glu Thr Cys Tyr Phe Val Glu Ala Leu Ile Lys
Cys Asn Leu Lys Ser Leu Ala Asp Val Ser Glu Arg Leu Ala Ile Gln
Asp Arg Thr Glu Pro Ile Asp Arg Met
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<210> SEQ ID NO 38
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<212> TYPE: PRT
<213> ORGANISM: Vitis vinifera
<220> FEATURE:
<223> OTHER INFORMATION: grapevine cultivar PN40024 unnamed protein
     product, locus GSVIVT00006507001
<400> SEOUENCE: 38
Met Asn Gly Asn Gly Leu Ser Ser Met Glu Ser Glu Tyr Ile Arg Arg
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His His Arg His Glu Pro Ala Glu Asn Gln Cys Ser Ser Ala Leu Val
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Lys His Ile Lys Ala Pro Val Pro Leu Val Trp Ser Leu Val Arg Arg
                          40
Phe Asp Gln Pro Gln Lys Tyr Lys Pro Phe Ile Ser Arg Cys Val Val
Gln Gly Asn Leu Glu Ile Gly Ser Leu Arg Glu Val Asp Val Lys Ser
Gly Leu Pro Ala Thr Thr Ser Thr Glu Arg Leu Glu Leu Leu Asp Asp
Asp Glu His Ile Leu Ser Met Arg Ile Ile Gly Gly Asp His Arg Leu
Arg Asn Tyr Ser Ser Ile Ile Ser Leu His Pro Glu Ile Ile Asp Gly
Arg Pro Gly Thr Met Val Ile Glu Ser Tyr Val Val Asp Val Pro Glu
Gly Asn Thr Lys Asp Glu Thr Cys Tyr Phe Val Glu Ala Leu Ile Lys
Cys Asn Leu Lys Ser Leu Ala Asp Val Ser Glu Arg Leu Ala Val Gln
Asp Arg Thr Glu Pro Ile Asp Arg Met
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<210> SEQ ID NO 39
<211> LENGTH: 208
<212> TYPE: PRT
<213 > ORGANISM: Oryza sativa
<220> FEATURE:
<223> OTHER INFORMATION: rice Japonica cultivar group, cultivar
    Nipponbare hypothetical protein OsJ_21703
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<400> SEQUENCE: 39 Met Glu Ala His Val Glu Arg Ala Leu Arg Glu Gly Leu Thr Glu Glu Glu Arg Ala Ala Leu Glu Pro Ala Val Met Ala His His Thr Phe Pro 25 Pro Ser Thr Thr Thr Ala Thr Thr Ala Ala Ala Thr Cys Thr Ser Leu Val Thr Gln Arg Val Ala Ala Pro Val Arg Ala Val Trp Pro Ile Val Arg Ser Phe Gly Asn Pro Gln Arg Tyr Lys His Phe Val Arg Thr Cys Ala Leu Ala Ala Gly Asn Gly Pro Ser Phe Gly Ser Val Arg Glu Val Thr Val Val Ser Gly Pro Ser Arg Leu Pro Pro Gly Thr Glu Arg Leu Glu Met Leu Asp Asp Asp Arg His Ile Ile Ser Phe Arg Val Val Gly Gly Gln His Arg Leu Arg Asn Tyr Arg Ser Val Thr Ser Val Thr Glu 135 Phe Gln Pro Pro Ala Ala Gly Pro Gly Pro Ala Pro Pro Tyr Cys Val 150 155 Val Val Glu Ser Tyr Val Val Asp Val Pro Asp Gly Asn Thr Ala Glu 170 Asp Thr Arg Met Phe Thr Asp Thr Val Val Lys Leu Asn Leu Gln Met Leu Ala Ala Val Ala Glu Asp Ser Ser Ser Ala Ser Arg Arg Asp 200 <210> SEQ ID NO 40 <211> LENGTH: 186 <212> TYPE: PRT <213> ORGANISM: Capsicum annuum <220> FEATURE: <223> OTHER INFORMATION: pepper cultivar hanbyul CAPIP1 antimicrobial <400> SEQUENCE: 40 Met Met Asn Ala Asn Gly Phe Ser Gly Val Glu Lys Glu Tyr Ile Arg Lys His His Leu His Gln Pro Lys Glu Asn Gln Cys Ser Ser Phe Leu Val Lys His Ile Arg Ala Pro Val His Leu Val Trp Ser Leu Val Arg Arg Phe Asp Gln Pro Gln Lys Tyr Lys Pro Phe Val Ser Arg Cys Ile Ala Gln Gly Asp Leu Glu Ile Gly Ser Leu Arg Glu Val Asp Val Lys Ser Gly Leu Pro Ala Thr Thr Ser Thr Glu Arg Leu Glu Leu Leu Asp Asp Glu Glu His Ile Leu Ser Phe Arg Ile Ile Gly Gly Asp His Arg 105 Leu Arg Asn Tyr Ser Ser Ile Ile Ser Leu His Pro Glu Val Ile Asp 120 Gly Arg Pro Gly Thr Leu Val Ile Glu Ser Phe Val Val Asp Val Pro 135

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Gln Gly Asn Thr Lys Asp Glu Thr Cys Tyr Phe Val Glu Ala Leu Ile 155 Asn Cys Asn Leu Lys Ser Leu Ala Asp Val Ser Glu Arg Leu Ala Val Gln Asp Arg Thr Glu Pro Ile Asp Gln Val <210> SEQ ID NO 41 <211> LENGTH: 186 <212> TYPE: PRT <213 > ORGANISM: Populus trichocarpa <220> FEATURE: <223> OTHER INFORMATION: Western balsam poplar (black cottonwood) cultivar 383-2499 (Nisqually-1), clone PX0011\_H13 unknown protein <400> SEQUENCE: 41 Met Asn Gly Ser Asp Ala Tyr Ser Ala Thr Glu Ala Gln Tyr Val Arg 10 Arg His His Lys His Glu Pro Arg Glu Asn Gln Cys Thr Ser Ala Leu 25 Val Lys His Ile Lys Ala Pro Ala His Leu Val Trp Ser Leu Val Arg 40 Arg Phe Asp Gln Pro Gln Arg Tyr Lys Pro Phe Val Ser Arg Cys Val Met Asn Gly Glu Leu Gly Ile Gly Ser Val Arg Glu Val Asn Val Lys Ser Gly Leu Pro Ala Thr Thr Ser Thr Glu Arg Leu Glu Leu Leu Asp 85 Asp Glu Glu His Ile Leu Gly Val Gln Ile Val Gly Gly Asp His Arg Leu Lys Asn Tyr Ser Ser Ile Met Thr Val His Pro Glu Phe Ile Asp 120 Gly Arg Pro Gly Thr Leu Val Ile Glu Ser Phe Ile Val Asp Val Pro 135 Asp Gly Asn Thr Lys Asp Glu Thr Cys Tyr Phe Val Glu Ala Leu Ile 150 Arg Cys Asn Leu Lys Ser Leu Ala Asp Val Ser Glu Arg Met Ala Val Gln Asp Arg Val Glu Pro Val Asn Gln Phe 180 <210> SEQ ID NO 42 <211> LENGTH: 185 <212> TYPE: PRT <213 > ORGANISM: Capsicum annuum <220> FEATURE: <223> OTHER INFORMATION: pepper cultivar hanbyul PIP1 (CAPIP1) antimicrobial protein <400> SEQUENCE: 42 Met Asn Ala Asn Gly Phe Ser Gly Val Glu Lys Glu Tyr Ile Arg Lys 10 His His Leu His Gln Pro Lys Glu Asn Gln Cys Ser Ser Phe Leu Val Lys His Ile Arg Ala Pro Val His Leu Val Trp Ser Leu Val Arg Arg 40 Phe Asp Gln Pro Gln Lys Tyr Lys Pro Phe Val Ser Arg Cys Ile Ala

	50					55					60				
Gln 65	Gly	Asp	Leu	Glu	Ile 70	Gly	Ser	Leu	Arg	Glu 75	Val	Asp	Val	ГЛа	Ser 80
Gly	Leu	Pro	Ala	Thr 85	Thr	Ser	Thr	Glu	Arg 90	Leu	Glu	Leu	Leu	Asp 95	Asp
Glu	Glu	His	Ile 100	Leu	Ser	Phe	Arg	Ile 105	Ile	Gly	Gly	Asp	His 110	Arg	Leu
Arg	Asn	Tyr 115	Ser	Ser	Ile	Ile	Ser 120	Leu	His	Pro	Glu	Val 125	Ile	Asp	Gly
Arg	Pro 130	Gly	Thr	Leu	Val	Ile 135	Glu	Ser	Phe	Val	Val 140	Asp	Val	Pro	Gln
Gly 145	Asn	Thr	Lys	Asp	Glu 150	Thr	Cys	Tyr	Phe	Val 155	Glu	Ala	Leu	Ile	Asn 160
Cys	Asn	Leu	Lys	Ser 165	Leu	Ala	Asp	Val	Ser 170	Glu	Arg	Leu	Ala	Val 175	Gln
Asp	Arg	Thr	Glu 180	Pro	Ile	Asp	Gln	Val 185							
<210 <211 <212 <213 <220 <223	> LH > TY > OH > FH > OT Ea	ENGTH (PE: RGAN] EATUR THER aster	H: 19 PRT SM: RE: INFO	36 Popi DRMA:	TION nwood	: Wes	steri	-	lsam	pop:	lar	(bla	ck c		nwood) x
< 400	> SI	EQUE	ICE :	43											
Met 1	Asn	Gly	Ser	Asp 5	Ala	Tyr	Ser	Ala	Thr 10	Glu	Ala	Gln	Tyr	Val 15	Arg
Arg	His	His	Lys 20	His	Glu	Pro	Arg	Glu 25	Asn	Gln	Сув	Thr	Ser 30	Ala	Leu
Val	Lys	His 35	Ile	Lys	Ala	Pro	Ala 40	His	Leu	Val	Trp	Ser 45	Leu	Val	Arg
Arg	Phe 50	Asp	Gln	Pro	Gln	Arg 55	Tyr	Lys	Pro	Phe	Val 60	Ser	Arg	CÀa	Val
Met 65	Asn	Gly	Glu	Leu	Gly 70	Ile	Gly	Ser	Val	Arg 75	Glu	Val	Asn	Val	80 TÀa
Ser	Gly	Leu	Pro	Ala 85	Thr	Thr	Ser	Thr	Glu 90	Arg	Leu	Glu	Leu	Leu 95	Asp
Asp	Glu	Glu	His 100	Ile	Leu	Gly	Val	Gln 105	Ile	Val	Gly	Gly	Asp 110	His	Arg
Leu	Lys	Asn 115	Tyr	Ser	Ser	Ile	Met 120	Thr	Val	His	Pro	Glu 125	Phe	Ile	Asp
Gly	Arg 130	Pro	Gly	Thr	Leu	Val 135	Ile	Glu	Ser	Phe	Ile 140	Val	Asp	Val	Pro
Asp 145	Gly	Asn	Thr	Lys	Asp 150	Glu	Thr	Cys	Tyr	Phe 155	Val	Lys	Ala	Leu	Ile 160
Arg	Cya	Asn	Leu	Lys 165	Ser	Leu	Ala	Asp	Val 170	Ser	Glu	Arg	Met	Ala 175	Val
Gln	Asp	Arg	Val 180	Glu	Pro	Val	Asn	Gln 185	Phe						
<210 <211 <212 <213	> LE > T	ENGTI (PE :	H: 19 PRT	38	ım sa	ativ	ım								

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<223> OTHER INFORMATION: pea AT-rich element binding factor 3 (ATF3,
     PsATF), potential transcription factor for PsCHS1
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Arg Arg Arg His Lys His Asp Leu Arg Asp Asn Gln Cys Ser Ser Ala
Leu Val Lys His Ile Lys Ala Pro Val His Leu Val Trp Ser Leu Val
Arg Arg Phe Asp Gln Pro Gln Lys Tyr Lys Pro Phe Val Ser Arg Cys
Ile Met Gln Gly Asp Leu Gly Ile Gly Ser Val Arg Glu Val Asn Val
Lys Ser Gly Leu Pro Ala Thr Thr Ser Thr Glu Arg Leu Glu Gln Leu
Asp Asp Glu Glu His Ile Leu Gly Ile Arg Ile Val Gly Gly Asp His 100 $100$
Arg Leu Arg Asn Tyr Ser Ser Val Ile Thr Val His Pro Glu Val Ile
Asp Gly Arg Pro Gly Thr Met Val Ile Glu Ser Phe Val Val Asp Val
  130 135
Pro Glu Gly Asn Thr Arg Asp Glu Thr Cys Tyr Phe Val Glu Ala Leu
           150
                                    155
Ile Arg Gly Asn Leu Ser Ser Leu Ala Asp Val Ser Glu Arg Met Ala
Val Gln Gly Arg Thr Asp Pro Ile Asn Val Asn Pro
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<210> SEQ ID NO 45
<211> LENGTH: 177
<212> TYPE: PRT
<213> ORGANISM: Vitis vinifera
<220> FEATURE:
<223> OTHER INFORMATION: grapevine cultivar PN40024 unnamed protein
     product, locus GSVIVT00027009001
<400> SEQUENCE: 45
Met Glu Ala Gln Val Ile Cys Arg His His Ala His Glu Pro Arg Glu
Asn Gln Cys Ser Ser Val Leu Val Arg His Val Lys Ala Pro Ala Asn
Leu Val Trp Ser Leu Val Arg Arg Phe Asp Gln Pro Gln Lys Tyr Lys
Pro Phe Val Ser Arg Cys Val Val Gln Gly Asp Leu Arg Ile Gly Ser
Val Arg Glu Val Asn Val Lys Thr Gly Leu Pro Ala Thr Thr Ser Thr
Glu Arg Leu Glu Leu Phe Asp Asp Asp Glu His Val Leu Gly Ile Lys
Ile Leu Asp Gly Asp His Arg Leu Arg Asn Tyr Ser Ser Val Ile Thr
                             105
Val His Pro Glu Ile Ile Asp Gly Arg Pro Gly Thr Leu Val Ile Glu
                          120
                                              125
Ser Phe Val Val Asp Val Pro Glu Gly Asn Thr Lys Asp Asp Thr Cys
             135
                                 140
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Tyr Phe Val Arg Ala Leu Ile Asn Cys Asn Leu Lys Cys Leu Ala Glu 155 150 Val Ser Glu Arg Met Ala Met Leu Gly Arg Val Glu Pro Ala Asn Ala 165 170 Val <210> SEQ ID NO 46 <211> LENGTH: 178 <212> TYPE: PRT <213> ORGANISM: Vitis vinifera <220> FEATURE: <223> OTHER INFORMATION: grapevine cultivar Pinot Noir, clone ENTAV 115 hypothetical protein, locus VITISV\_004915 <400> SEQUENCE: 46 Met Met Glu Ala Gln Val Ile Cys Arg His His Ala His Glu Pro Arg Glu Asn Gln Cys Ser Ser Val Leu Val Arg His Val Lys Ala Pro Ala Asn Leu Val Trp Ser Leu Val Arg Arg Phe Asp Gln Pro Gln Lys Tyr 40 Lys Pro Phe Val Ser Arg Cys Val Val Gln Gly Asp Leu Arg Ile Gly Ser Val Arg Glu Val Asn Val Lys Thr Gly Leu Pro Ala Thr Thr Ser Thr Glu Arg Leu Glu Leu Phe Asp Asp Glu His Val Leu Gly Ile Lys Ile Leu Asp Gly Asp His Arg Leu Arg Asn Tyr Ser Ser Val Ile 105 Thr Val His Pro Glu Ile Ile Asp Gly Arg Pro Gly Thr Leu Val Ile 120 Glu Ser Phe Val Val Asp Val Pro Glu Gly Asn Thr Lys Asp Asp Thr 135 Cys Tyr Phe Val Arg Ala Leu Ile Asn Cys Asn Leu Lys Cys Leu Ala 150 155 Glu Val Ser Glu Arg Met Ala Met Leu Gly Arg Val Glu Pro Ala Asn Ala Val <210> SEQ ID NO 47 <211> LENGTH: 193 <212> TYPE: PRT <213 > ORGANISM: Arachis hypogaea <220> FEATURE: <223> OTHER INFORMATION: peanut pathogenesis-induced protein (PIP, AhPIP) <221> NAME/KEY: VARIANT <222> LOCATION: (162) ... (162) <223> OTHER INFORMATION: Xaa = Asp, ASn, Tyr or His <400> SEQUENCE: 47 Met Met Asn Gly Ser Cys Gly Gly Gly Gly Gly Glu Ala Tyr Gly 1.0 Ala Ile Glu Ala Gln Tyr Ile Arg Arg His His Arg His Glu Pro Arg Asp Asn Gln Cys Thr Ser Ala Leu Val Lys His Ile Arg Ala Pro Val 40 His Leu Val Trp Ser Leu Val Arg Arg Phe Asp Gln Pro Gln Lys Tyr

	50					55					60				
Lys 65	Pro	Phe	Val	Ser	Arg 70	Cys	Ile	Met	Gln	Gly 75	Asp	Leu	Gly	Ile	Gly 80
Ser	Val	Arg	Glu	Val 85	Asn	Val	Lys	Ser	Gly 90	Leu	Pro	Ala	Thr	Thr 95	Ser
Thr	Glu	Arg	Leu 100	Glu	Gln	Leu	Asp	Asp 105	Glu	Glu	His	Ile	Leu 110	Gly	Ile
Arg	Ile	Val 115	Gly	Gly	Asp	His	Arg 120	Leu	Arg	Asn	Tyr	Ser 125	Ser	Ile	Ile
Thr	Val 130	His	Pro	Glu	Val	Ile 135	Glu	Gly	Arg	Pro	Gly 140	Thr	Met	Val	Ile
Glu 145	Ser	Phe	Val	Val	Asp 150	Val	Pro	Asp	Gly	Asn 155	Thr	Lys	Asp	Glu	Thr 160
Cys	Xaa	Phe	Val	Glu 165	Ala	Leu	Ile	Arg	Cys 170	Asn	Leu	Ser	Ser	Leu 175	Ala
Asp	Val	Ser	Glu 180	Arg	Met	Ala	Val	Gln 185	Gly	Arg	Thr	Asp	Pro 190	Ile	Asn
Gln															
<211	> LI	EQ II ENGTI YPE:	H: 2												
<213	3 > OF	RGAN:	ISM:	Zea	may	3									
	3 > 07	EATUI CHER indi:	INF			: coi	rn (t	maize	∍), ‹	clone	e 300	0908	AT-1	rich	element
< 400	)> SI	EQUEI	ICE :	48											
Met 1	Val	Val	Glu	Met 5	Asp	Gly	Gly	Val	Gly 10	Val	Ala	Ala	Gly	Gly 15	Gly
Gly	Gly	Ala	Gln 20	Thr	Pro	Ala	Pro	Ala 25	Pro	Pro	Arg	Arg	Trp 30	Arg	Leu
Ala	Asp	Glu 35	Arg	Сув	Asp	Leu	Arg 40	Ala	Met	Glu	Thr	Asp 45	Tyr	Val	Arg
Arg	Phe 50	His	Arg	His	Glu	Pro 55	Arg	Asp	His	Gln	Cys	Ser	Ser	Ala	Val
Ala 65	Lys	His	Ile	Lys	Ala 70	Pro	Val	His	Leu	Val 75	Trp	Ser	Leu	Val	Arg 80
Arg	Phe	Asp	Gln	Pro 85	Gln	Leu	Phe	-	Pro 90	Phe	Val	Ser	Arg	Сув 95	Glu
Met	Lys	Gly	Asn 100	Ile	Glu	Ile	Gly	Ser 105	Val	Arg	Glu	Val	Asn 110	Val	Lys
Ser	Gly	Leu 115	Pro	Ala	Thr	Arg	Ser 120	Thr	Glu	Arg	Leu	Glu 125	Leu	Leu	Asp
Asp	Asp 130	Glu	Arg	Ile	Leu	Ser 135	Val	Arg	Phe	Val	Gly 140	Gly	Asp	His	Arg
Leu 145	Gln	Asn	Tyr	Ser	Ser 150	Ile	Leu	Thr	Val	His 155	Pro	Glu	Val	Ile	Asp 160
Gly	Arg	Pro	Gly	Thr 165	Leu	Val	Ile	Glu	Ser 170	Phe	Val	Val	Asp	Val 175	Pro
Asp	Gly	Asn	Thr 180	Lys	Asp	Glu	Thr	Cys 185	Tyr	Phe	Val	Glu	Ala 190	Leu	Leu
Lys	Cys	Asn 195	Leu	Arg	Ser	Leu	Ala 200	Glu	Val	Ser	Glu	Gly 205	Gln	Val	Ile
Met	Asp	Gln	Thr	Glu	Pro	Leu	Asp	Arg							

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<210> SEQ ID NO 49
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<212> TYPE: PRT
<213> ORGANISM: Zea mays
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<223> OTHER INFORMATION: corn (maize) strain B73, clone ZM_BFb0036A01
     unknown protein
<400> SEQUENCE: 49
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Gly Gly Ala Gln Thr Pro Ala Pro Pro Pro Pro Arg Arg Trp Arg Leu
Ala Asp Glu Arg Cys Asp Leu Arg Ala Met Glu Thr Asp Tyr Val Arg
Arg Phe His Arg His Glu Pro Arg Asp His Gln Cys Ser Ser Ala Val
          55
Ala Lys His Ile Lys Ala Pro Val His Leu Val Trp Ser Leu Val Arg
                                     75
                 70
Arg Phe Asp Gln Pro Gln Leu Phe Lys Pro Phe Val Ser Arg Cys Glu
Met Lys Gly Asn Ile Glu Ile Gly Ser Val Arg Glu Val Asn Val Lys
                              105
Ser Gly Leu Pro Ala Thr Arg Ser Thr Glu Arg Leu Glu Leu Leu Asp
                  120
Asp Asp Glu Arg Ile Leu Ser Val Arg Phe Val Gly Gly Asp His Arg
                     135
Leu Gln Asn Tyr Ser Ser Ile Leu Thr Val His Pro Glu Val Ile Asp
                  150
Gly Arg Pro Gly Thr Leu Val Ile Glu Ser Phe Val Val Asp Val Pro
                        170
Asp Gly Asn Thr Lys Asp Glu Thr Cys Tyr Phe Val Glu Ala Leu Leu
                            185
Lys Cys Asn Leu Arg Ser Leu Ala Glu Val Ser Glu Gly Gln Val Ile
                         200
Met Asp Gln Thr Glu Pro Leu Asp Arg
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<211> LENGTH: 206
<212> TYPE: PRT
<213> ORGANISM: Oryza sativa
<220> FEATURE:
<223> OTHER INFORMATION: rice Japonica cultivar group, cultivar
     Nipponbare (GA3) conserved hypothetical protein 0s06g0528300
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                                  10
Met Val Ser His Arg Arg Val Gln Trp Arg Leu Ala Asp Glu Arg Cys
                              25
Glu Leu Arg Glu Glu Glu Met Glu Tyr Ile Arg Arg Phe His Arg His
Glu Pro Ser Ser Asn Gln Cys Thr Ser Phe Ala Ala Lys His Ile Lys
Ala Pro Leu His Thr Val Trp Ser Leu Val Arg Arg Phe Asp Gln Pro
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<u></u>		7.0				7.5					00
65		70				75					80
Gln Leu Ph	e Lys Pro 85	Phe Val	Arg	Asn	oo Gys	Val	Met	Arg	Glu	Asn 95	Ile
Ile Ala Th	r Gly Cys 100	Ile Arg	Glu	Val 105	Asn	Val	Gln	Ser	Gly 110	Leu	Pro
Ala Thr Ar		Glu Arg	Leu 120	Glu	Leu	Leu	Asp	Asp 125	Asn	Glu	His
Ile Leu Ly 130	s Val Asr	n Phe Ile 135		Gly	Asp	His	Met 140	Leu	Lys	Asn	Tyr
Ser Ser Il 145	e Leu Thi	Val His	Ser	Glu	Val	Ile 155	Asp	Gly	Gln	Leu	Gly 160
Thr Leu Va	l Val Glu 165		Ile	Val	Asp 170	Val	Pro	Glu	Gly	Asn 175	Thr
Lya Aap Aa	p Ile Ser 180	Tyr Phe	Ile	Glu 185	Asn	Val	Leu	Arg	Cys 190	Asn	Leu
Arg Thr Le		Val Ser	Glu 200	Glu	Arg	Leu	Ala	Asn 205	Pro		
<210> SEQ <211> LENG <212> TYPE <213> ORGA <220> FEAT <223> OTHE hypo	TH: 206 : PRT NISM: Ory URE:	ATION: ri	ce In			ltiva	ar g:	roup	, cul	ltiva	ar 93-11
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Met Asn Gl 1	y Ala Gly 5	Gly Ala	Gly	Gly	Ala 10	Ala	Ala	Gly	Lys	Leu 15	Pro
Met Val Se	r His Arç 20	g Gln Val	Gln	Trp 25	Arg	Leu	Ala	Asp	Glu 30	Arg	Cys
Glu Leu Ar 35	g Glu Glu	ı Glu Met	Glu 40	Tyr	Ile	Arg	Gln	Phe 45	His	Arg	His
Glu Pro Se 50	r Ser Asr	n Gln Cys 55	Thr	Ser	Phe	Val	Ala 60	ГÀа	His	Ile	ГЛа
Ala Pro Le 65	u Gln Thi	Val Trp 70	Ser	Leu	Val	Arg 75	Arg	Phe	Asp	Gln	Pro 80
Gln Leu Ph	e Lys Pro 85	Phe Val	Arg	Lys	Сув 90	Val	Met	Arg	Glu	Asn 95	Ile
Ile Ala Th	r Gly Cys 100	Val Arg		Val 105	Asn	Val	Gln	Ser	Gly 110	Leu	Pro
Ala Thr Ar 11		Glu Arg	Leu 120	Glu	Leu	Leu	Asp	Asp 125	Asn	Glu	His
Ile Leu Ly 130	s Val Lys	Phe Ile 135		Gly	Asp	His	Met 140	Leu	ГÀа	Asn	Tyr
Ser Ser Il 145	e Leu Thi	: Ile His 150	Ser	Glu	Val	Ile 155	Asp	Gly	Gln	Leu	Gly 160
Thr Leu Va	l Val Glu 165		Val		Asp 170	Ile	Pro	Glu	Gly	Asn 175	Thr
Lys Asp As	p Ile Cys 180	Tyr Phe		Glu 185	Asn	Ile	Leu	Arg	Cys 190	Asn	Leu
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<210> SEQ ID NO 52 <211> LENGTH: 205

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<220> FEATURE:
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Trp Arg Leu Ala Asp Glu Arg Cys Asp Leu Arg Ala Ala Glu Thr Glu
Tyr Val Arg Arg Phe His Arg His Glu Pro Arg Asp His Gln Cys Ser
Ser Ala Val Ala Lys His Ile Lys Ala Pro Val His Leu Val Trp Ser
Leu Val Arg Arg Phe Asp Gln Pro Gln Leu Phe Lys Pro Phe Val Ser
 \hbox{Arg Cys Glu Met Lys Gly Asn Ile Glu Ile Gly Ser Val Arg Glu Val} \\
Asn Val Lys Ser Gly Leu Pro Ala Thr Arg Ser Thr Glu Arg Leu Glu
                              105
Leu Leu Asp Asp Asn Glu His Ile Leu Ser Val Arg Phe Val Gly Gly
                         120
Asp His Arg Leu Lys Asn Tyr Ser Ser Ile Leu Thr Val His Pro Glu
Val Ile Asp Gly Arg Pro Gly Thr Leu Val Ile Glu Ser Phe Val Val
                            155
Asp Val Pro Glu Gly Asn Thr Lys Asp Glu Thr Cys Tyr Phe Val Glu
                         170
Ala Leu Leu Lys Cys Asn Leu Lys Ser Leu Ala Glu Val Ser Glu Arg
                              185
Leu Val Cys Gln Gly Pro Asn Arg Ala Pro Ser Thr Arg
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<211> LENGTH: 204
<212> TYPE: PRT
<213> ORGANISM: Oryza sativa
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<223> OTHER INFORMATION: rice Japonica cultivar group, cultivar
     Nipponbare (GA3) conserved hypothetical protein 0s02g0255500
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Trp Arg Leu Ala Asp Glu Arg Cys Asp Leu Arg Ala Ala Glu Thr Glu 20 25 30
Tyr Val Arg Arg Phe His Arg His Glu Pro Arg Asp His Gln Cys Ser
                           40
Ser Ala Val Ala Lys His Ile Lys Ala Pro Val His Leu Val Trp Ser
Leu Val Arg Arg Phe Asp Gln Pro Gln Leu Phe Lys Pro Phe Val Ser
Arg Cys Glu Met Lys Gly Asn Ile Glu Ile Gly Ser Val Arg Glu Val
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Asn Val Lys Ser Gly Leu Pro Ala Thr Arg Ser Thr Glu Arg Leu Glu 100 105 110

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Leu Leu Asp Asp Asn Glu His Ile Leu Ser Val Arg Phe Val Gly Gly
       115
                           120
Asp His Arg Leu Lys Asn Tyr Ser Ser Ile Leu Thr Val His Pro Glu
Val Ile Asp Gly Arg Pro Gly Thr Leu Val Ile Glu Ser Phe Val Val
Asp Val Pro Glu Gly Asn Thr Lys Asp Glu Thr Cys Tyr Phe Val Glu
Ala Leu Leu Lys Cys Asn Leu Lys Ser Leu Ala Glu Val Ser Glu Arg
Leu Val Val Lys Asp Gln Thr Glu Pro Leu Asp Arg
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<213 > ORGANISM: Medicago truncatula
<220> FEATURE:
<223> OTHER INFORMATION: barrel medic unkown protein, clone
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Gln Cys Ser Ser Ala Leu Val Lys His Ile Arg Ala Pro Val Pro Leu
Val Trp Ser Leu Val Arg Arg Phe Asp Gln Pro Gln Lys Tyr Lys Pro
Phe Val Ser Arg Cys Val Val Arg Gly Asn Leu Glu Ile Gly Ser Leu
                   70
Arg Glu Val Asp Val Lys Ser Gly Leu Pro Ala Thr Thr Ser Thr Glu
Arg Leu Glu Val Leu Asp Asp Asn Glu His Ile Leu Ser Ile Arg Ile
                      105
Ile Gly Gly Asp His Arg Leu Arg Asn Tyr Ser Ser Ile Met Ser Leu
His Pro Glu Ile Ile Asp Gly Arg Pro Gly Thr Leu Val Ile Glu Ser
Phe Val Val Asp Val Pro Glu Gly Asn Thr Lys Asp Glu Thr Cys Tyr
Phe Val Glu Ala Leu Ile Lys Cys Asn Leu Lys Ser Leu Ser Asp Val
Ser Glu Gly His Ala Val Gln Asp Leu Thr Glu Pro Leu Asp Arg Val
                              185
His Glu Leu Leu Ile Ser Gly
       195
<210> SEQ ID NO 55
<211> LENGTH: 199
<212> TYPE: PRT
<213> ORGANISM: Medicago truncatula
<220> FEATURE:
<223> OTHER INFORMATION: barrel medic unkown protein, clone
     MTYF1_F2_F3_F41G-K-4
<400> SEQUENCE: 55
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Met Glu Lys Met Asn Gly Thr Glu Asn Asn Gly Val Phe Asn Ser Thr Glu Met Glu Tyr Ile Arg Arg His His Asn Gln Gln Pro Gly Glu Asn Gln Cys Ser Ser Ala Leu Val Lys His Ile Arg Ala Pro Val Pro Leu Val Trp Ser Leu Val Arg Arg Phe Asp Gln Pro Gln Lys Tyr Lys Pro Phe Val Ser Arg Cys Val Val Arg Gly Asn Leu Glu Ile Gly Ser Leu  $\hbox{Arg Glu Val Asp Val Lys Ser Gly Leu Pro Ala Thr Thr Ser Thr Glu } \\$ Arg Leu Glu Val Leu Asp Asp Asn Glu His Ile Leu Ser Ile Arg Ile Ile Gly Gly Asp His Arg Leu Arg Asn Tyr Ser Ser Ile Met Ser Leu 115 120 125 His Pro Glu Ile Ile Asp Gly Arg Pro Gly Thr Leu Val Ile Glu Ser 130 135 140 Phe Val Val Asp Val Pro Glu Gly Asn Thr Lys Asp Glu Thr Cys Tyr 155 Phe Val Glu Ala Leu Ile Lys Cys Asn Leu Lys Ser Leu Ser Asp Val 170 Ser Glu Gly His Ala Ala Gln Asp Leu Thr Glu Pro Leu Asp Arg Met 180 185 His Glu Leu Leu Ile Ser Gly 195 <210> SEO ID NO 56 <211> LENGTH: 197 <212> TYPE: PRT <213> ORGANISM: Zea mays <220> FEATURE: <223> OTHER INFORMATION: corn (maize), clone 244179 CAPIP1 antimicrobial protein <400> SEQUENCE: 56 Met Val Gly Leu Val Gly Gly Ser Thr Ala Arg Ala Glu His Val Val Ala Asn Ala Gly Gly Glu Ala Glu Tyr Val Arg Arg Met His Arg His Ala Pro Thr Glu His Gln Cys Thr Ser Thr Leu Val Lys His Ile Lys Ala Pro Val His Leu Val Trp Gln Leu Val Arg Arg Phe Asp Gln Pro 50 60 Gln Arg Tyr Lys Pro Phe Val Arg Asn Cys Val Val Arg Gly Asp Gln Leu Glu Val Gly Ser Leu Arg Asp Val Asn Val Lys Thr Gly Leu Pro Ala Thr Thr Ser Thr Glu Arg Leu Glu Gln Leu Asp Asp Asp Leu His 100 105 Ile Leu Gly Val Lys Phe Val Gly Gly Asp His Arg Leu Gln Asn Tyr 120 Ser Ser Ile Ile Thr Val His Pro Glu Ser Ile Asp Gly Arg Pro Gly 135 140 Thr Leu Val Ile Glu Ser Phe Val Val Asp Val Pro Asp Gly Asn Thr 150 155

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Lys Asp Glu Thr Cys Tyr Phe Val Glu Ala Val Ile Lys Cys Asn Leu 170 Asn Ser Leu Ala Glu Val Ser Glu Gln Leu Ala Val Glu Ser Pro Thr 180 185 Ser Leu Ile Asp Gln 195 <210> SEQ ID NO 57 <211> LENGTH: 197 <212> TYPE: PRT <213> ORGANISM: Zea mays <220> FEATURE: <223> OTHER INFORMATION: corn (maize), clone 1448906 CAPIP1 antimicrobial protein <400> SEQUENCE: 57 Met Val Gly Leu Val Gly Gly Ser Thr Ala Arg Ala Glu His Val Val Ala Asn Ala Gly Gly Glu Ala Glu Tyr Val Arg Arg Met His Arg His 20 25 30Ala Pro Thr Glu His Gln Cys Thr Ser Thr Leu Val Lys His Ile Lys Ala Pro Val His Leu Val Trp Glu Leu Val Arg Arg Phe Asp Gln Pro 55 Gln Arg Tyr Lys Pro Phe Val Arg Asn Cys Val Val Arg Gly Asp Gln 70 Leu Glu Val Gly Ser Leu Arg Asp Val Asn Val Lys Thr Gly Leu Pro Ala Thr Thr Ser Thr Glu Arg Leu Glu Gln Leu Asp Asp Leu His 105 Ile Leu Gly Val Lys Phe Val Gly Gly Asp His Arg Leu Gln Asn Tyr 120 Ser Ser Ile Ile Thr Val His Pro Glu Ser Ile Asp Gly Arg Pro Gly 135 Thr Leu Val Ile Glu Ser Phe Val Val Asp Val Pro Asp Gly Asn Thr Lys Asp Glu Thr Cys Tyr Phe Val Glu Ala Val Ile Lys Cys Asn Leu 170 Asn Ser Leu Ala Glu Val Ser Glu Gln Leu Ala Val Glu Ser Pro Thr Ser Leu Ile Asp Gln 195 <210> SEQ ID NO 58 <211> LENGTH: 212 <212> TYPE: PRT <213 > ORGANISM: Zea mays <220> FEATURE: <223> OTHER INFORMATION: corn (maize) strain B73, clone ZM\_BFc0183D21 unknown protein <400> SEQUENCE: 58 Met Val Met Val Glu Met Asp Gly Gly Val Gly Gly Gly Gly Gly 10 Gly Gln Thr Pro Ala Pro Arg Arg Trp Arg Leu Ala Asp Glu Arg Cys 25 Asp Leu Arg Ala Met Glu Thr Asp Tyr Val Arg Arg Phe His Arg His 40

Glu Pro Arg													
50	Glu	His	Gln	Сув 55	Ser	Ser	Ala	Val	Ala 60	Lys	His	Ile	ГЛа
Ala Pro Val 65	His	Leu	Val 70	Trp	Ser	Leu	Val	Arg 75	Arg	Phe	Asp	Gln	Pro 80
Gln Leu Phe	Lys	Pro 85	Phe	Val	Ser	Arg	Cys 90	Glu	Met	Lys	Gly	Asn 95	Ile
Glu Ile Gly	Ser 100	Val	Arg	Glu	Val	Asn 105	Val	Lys	Ser	Gly	Leu 110	Pro	Ala
Thr Arg Ser	Thr	Glu	Arg	Leu	Glu 120	Leu	Leu	Asp	Asp	Asn 125	Glu	His	Ile
Leu Ser Val	Arg	Phe	Val	Gly 135	Gly	Asp	His	Arg	Leu 140	Gln	Asn	Tyr	Ser
Ser Ile Leu 145	Thr	Val	His 150	Pro	Glu	Val	Ile	Asp 155	Gly	Arg	Pro	Gly	Thr 160
Leu Val Ile	Glu	Ser 165	Phe	Val	Val	Asp	Val 170	Pro	Asp	Gly	Asn	Thr 175	Lya
Asp Glu Thr	Cys 180	Tyr	Phe	Val	Glu	Ala 185	Leu	Leu	Lys	Cys	Asn 190	Leu	Lya
Ser Leu Ala 195	Glu	Val	Ser	Glu	Arg 200	Gln	Val	Val	Lys	Asp 205	Gln	Thr	Glu
Pro Leu Asp 210	Arg												
<213> ORGAN <220> FEATU <223> OTHER	RE:	_				non	ica (	]+:	iver	~~~	n (	] + i	
Nippo			43) (	conse									)527800
	NCE :	59			erve	l hyp	othe	etica	al pi	rote	in Os	306g(	)527800
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<pre>&lt;400&gt; SEQUE Met Asn Gly 1 Met Val Ser Glu Leu Arg 35 Glu Pro Ser</pre>	NCE: Ala His 20 Glu Ser	59 Gly 5 Arg Glu Asn	Gly Arg Glu Gln	Ala Val Met Cys 55	Gly Gln Glu 40	Gly Cys 25 Tyr	Ala 10 Arg Ile	Ala Leu Arg	Ala Ala Gln Ala 60	Gly Asp Phe 45	Lys Lys 30 His	Leu 15 Arg Arg	Pro Cys His
<pre>&lt;400&gt; SEQUE Met Asn Gly 1 Met Val Ser Glu Leu Arg 35 Glu Pro Ser 50</pre>	NCE: Ala His 20 Glu Ser Gln	59 Gly 5 Arg Glu Asn Thr	Gly Arg Glu Gln Val	Ala Val Met Cys 55 Trp	Gly Gln Glu 40 Thr	Gly Cys 25 Tyr Ser	Ala 10 Arg Ile Phe	Ala Leu Arg Val	Ala Ala Gln Ala 60 Arg	Gly Asp Phe 45 Lys	Lys Lys 30 His Asp	Leu 15 Arg Arg Ile	Pro Cys His Lys Pro 80
<pre>&lt;400&gt; SEQUE Met Asn Gly 1 Met Val Ser Glu Leu Arg 35 Glu Pro Ser 50 Ala Pro Leu 65</pre>	NCE: Ala His 20 Glu Ser Gln Lys	Gly 5 Arg Glu Asn Thr	Gly Arg Glu Gln Val 70 Phe	Ala Val Met Cys 55 Trp Val	Gly Gln Glu 40 Thr Ser	Gly Cys 25 Tyr Ser Leu	Ala 10 Arg Ile Phe Val Cys 90	Ala Leu Arg Val Arg 75	Ala Ala Gln Ala 60 Arg	Gly Asp Phe 45 Lys Phe Arg	Lys Lys 30 His Asp	Leu 15 Arg Arg Ile Gln Asn 95	Pro Cys His Lys Pro 80
<pre>&lt;400&gt; SEQUE Met Asn Gly 1 Met Val Ser Glu Leu Arg 35 Glu Pro Ser 50 Ala Pro Leu 65 Gln Leu Phe</pre>	NCE: Ala His 20 Glu Ser Gln Lys Gly 100	Gly 5 Arg Glu Asn Thr Cys	Gly Arg Glu Gln Val 70 Phe	Ala Val Met Cys 55 Trp Val Arg	Gly Glu 40 Thr Ser Arg	Gly Cys 25 Tyr Ser Leu Lys Val	Ala 10 Arg Ile Phe Val Cys 90 Asn	Ala Leu Arg Val Arg 75 Val	Ala Ala Gln Ala 60 Arg Met	Gly Asp Phe 45 Lys Phe Arg	Lys Lys 30 His Asp Glu Gly 110	Leu 15 Arg Arg Ile Gln Asn 95 Leu	Pro Cys His Lys Pro 80 Ile
<pre>&lt;400&gt; SEQUE Met Asn Gly 1 Met Val Ser Glu Leu Arg 35 Glu Pro Ser 50 Ala Pro Leu 65 Gln Leu Phe Ile Val Thr Ala Thr Arg</pre>	NCE: Ala His 20 Glu Ser Gln Lys Gly 100 Ser	Gly 5 Arg Glu Asn Thr Cys Thr	Gly Arg Glu Gln Val 70 Phe Val	Ala Val Met Cys 55 Trp Val Arg	Gly Gln Glu 40 Thr Ser Arg Glu Leu 120	Gly Cys 25 Tyr Ser Leu Lys Val 105 Glu	Ala 10 Arg Ile Phe Val Cys 90 Asn	Ala Leu Arg Val Arg 75 Val Val	Ala Ala Gln Arg Met Gln Asp	Gly Asp Phe 45 Lys Phe Arg Ser Asp 125	Lys Lys 30 His Asp Glu Gly 110 Asn	Leu 15 Arg Arg Ile Gln Asn 95 Leu	Pro Cys His Lys Pro 80 Ile Pro
<pre>&lt;400&gt; SEQUE Met Asn Gly 1 Met Val Ser Glu Leu Arg 35 Glu Pro Ser 50 Ala Pro Leu 65 Gln Leu Phe Ile Val Thr Ala Thr Arg 115 Ile Leu Lys</pre>	NCE: Ala His 20 Glu Ser Gln Lys Gly 100 Ser Val	Gly 5 Arg Glu Asn Thr Cys Thr	Gly Arg Glu Gln Val 70 Phe Val Glu	Ala Val Met Cys 55 Trp Val Arg Arg	Gly Gln Glu 40 Thr Ser Arg Glu Leu 120 Gly	Gly Cys 25 Tyr Ser Leu Lys Glu Gly	Ala 10 Arg Ile Phe Val Cys 90 Asn Leu Asp	Ala Leu Arg Val Arg T5 Val Leu His	Ala Ala Gln Ala 60 Arg Met Gln Asp	Gly Asp Phe 45 Lys Phe Arg Ser Asp 125 Leu	Lys Lys 30 His Asp Glu Gly 110 Asn	Leu 15 Arg Arg Ile Gln Asn 95 Leu Asn	Pro Cys His Lys Pro 80 Ile Pro His
<pre>&lt;400&gt; SEQUE Met Asn Gly 1 Met Val Ser Glu Leu Arg 35 Glu Pro Ser 50 Ala Pro Leu 65 Gln Leu Phe Ile Val Thr Ala Thr Arg 115 Ile Leu Lys 130 Ser Ser Ile</pre>	NCE: Ala His 20 Glu Ser Gln Lys Gly 100 Ser Val Leu	Gly 5 Arg Glu Asn Thr Pro 85 Cys Thr Lys	Gly Arg Glu Gln Val 70 Phe Val Glu Fle Tle 150	Ala Val Met Cys 55 Trp Val Arg Arg His	Gly Gln Glu 40 Thr Ser Arg Glu Leu 120 Gly Ser	Gly Cys 25 Tyr Ser Leu Lys Val 105 Glu Gly Glu	Ala 10 Arg Ile Phe Val Cys 90 Asn Leu Asp	Ala Leu Arg Val Arg 75 Val Val Leu His	Ala Ala Gln Ala 60 Arg Met Gln Asp Met 140	Gly Asp Phe 45 Lys Phe Arg Ser Asp 125 Leu Gly	Lys Lys 30 His Asp Glu Gly 110 Asn Lys Gln	Leu 15 Arg Arg Ile Gln Asn 95 Leu Glu	Pro Cys His Lys Pro 80 Ile Pro His Tyr Gly 160

											_	con	tin	ued	
			180					185					190		
Met	Thr	Leu 195	Ala	Asp	Val	Ser	Glu 200	Glu	Arg	Leu	Ala	Asn 205	Pro		
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< 400	)> SI	EQUEI	ICE :	60											
Met 1	Val	Gly	Leu	Val 5	Gly	Gly	Ser	Thr	Ala 10	Arg	Ala	Glu	His	Val 15	Val
Ala	Asn	Ala	Gly 20	Gly	Glu	Thr	Glu	Tyr 25	Val	Arg	Arg	Leu	His 30	Arg	His
Ala	Pro	Ala 35	Glu	His	Gln	CAa	Thr 40	Ser	Thr	Leu	Val	Lуs 45	His	Ile	Lys
Ala	Pro 50	Val	His	Leu	Val	Trp 55	Glu	Leu	Val	Arg	Ser 60	Phe	Asp	Gln	Pro
Gln 65	Arg	Tyr	Lys	Pro	Phe 70	Val	Arg	Asn	CAa	Val 75	Val	Arg	Gly	Asp	Gln 80
Leu	Glu	Val	Gly	Ser 85	Leu	Arg	Asp	Val	Asn 90	Val	Lys	Thr	Gly	Leu 95	Pro
Ala	Thr	Thr	Ser 100	Thr	Glu	Arg	Leu	Glu 105	Gln	Leu	Asp	Asp	Asp 110	Leu	His
Ile	Leu	Gly 115	Val	Lys	Phe	Val	Gly 120	Gly	Asp	His	Arg	Leu 125	Gln	Asn	Tyr
Ser	Ser 130	Ile	Ile	Thr	Val	His 135	Pro	Glu	Ser	Ile	Asp 140	Gly	Arg	Pro	Gly
Thr 145	Leu	Val	Ile	Glu	Ser 150	Phe	Val	Val	Asp	Val 155	Pro	Asp	Gly	Asn	Thr 160
ГÀЗ	Asp	Glu	Thr	Cys 165	Tyr	Phe	Val	Glu	Ala 170	Val	Ile	ГÀа	Cha	Asn 175	Leu
Lys	Ser	Leu	Ala 180	Glu	Val	Ser	Glu	Gln 185	Leu	Ala	Val	Glu	Ser 190	Pro	Thr
Ser	Pro	Ile 195	Asp	Gln											
<213 <213 <213 <220		ENGTI (PE : RGAN: EATUI THER	H: 20 PRT ISM: RE: INFO	Ory: Ory: ORMA:	rion	: ri				ltiva	ar gi	roup	, cui	ltiva	ar 93-11
< 400	O> SI	EQUEI	ICE :	61											
Met 1	Asn	Gly	Val	Gly 5	Gly	Ala	Gly	Gly	Ala 10	Ala	Ala	Gly	Lys	Leu 15	Pro
Met	Val	Ser	His 20	Arg	Arg	Val	Gln	Trp 25	Arg	Leu	Ala	Asp	Glu 30	Arg	Сув
Glu	Leu	Arg 35	Glu	Glu	Glu	Met	Glu 40	Tyr	Ile	Arg	Arg	Phe 45	His	Arg	His
Glu	Pro 50	Ser	Ser	Asn	Gln	Cys 55	Thr	Ser	Phe	Ala	Ala 60	ГÀв	His	Ile	Lys
Ala	Pro	Leu	His	Thr	Val	Trp	Ser	Leu	Val	Arg	Arg	Phe	Asp	Gln	Pro

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65	70		75	80
Gln Leu Phe Lys	Pro Phe Val 85	Arg Asn Cys 90	Val Met Arg	Glu Asn Ile 95
Ile Ala Thr Gly 100		Glu Val Asn 105		Gly Leu Pro 110
Ala Thr Arg Ser 115	Thr Glu Arg	Leu Glu Leu 120	Leu Asp Asp 125	Asn Glu His
Ile Leu Lys Val 130	Lys Phe Ile 135	Gly Gly Asp	His Met Leu 140	Lys Asn Tyr
Ser Ser Ile Leu 145	Thr Val His 150	Ser Glu Val	Ile Asp Gly 155	Gln Leu Gly 160
Thr Leu Val Val	Glu Ser Phe 165	Ile Val Asp 170	Val Leu Glu	Gly Asn Thr 175
Lys Asp Asp Ile 180	Ser Tyr Phe	Ile Glu Asn 185		Cys Asn Leu 190
Arg Thr Leu Ala 195	Asp Val Ser	Glu Glu Arg 200	Leu Ala Asn 205	Pro
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<400> SEQUENCE:	62			
Met Val Gly Leu 1	Val Gly Gly 5	Gly Gly Trp 10	Arg Val Gly	Asp Asp Ala 15
Ala Gly Gly Gly 20	Gly Gly Gly	Ala Val Ala 25	_	Ala Ala Ala 30
Ala Glu Ala Glu 35	His Met Arg	Arg Leu His 40	Ser His Ala 45	Pro Gly Glu
His Gln Cys Ser 50	Ser Ala Leu 55	Val Lys His	Ile Lys Ala 60	Pro Val His
Leu Val Trp Ser 65	Leu Val Arg 70	Ser Phe Asp	Gln Pro Gln 75	Arg Tyr Lys 80
Pro Phe Val Ser	Arg Cys Val 85	Val Arg Gly 90	Gly Asp Leu	Glu Ile Gly 95
Ser Val Arg Glu 100	Val Asn Val	Lys Thr Gly 105		Thr Thr Ser 110
Thr Glu Arg Leu 115	Glu Leu Leu	Asp Asp Asp 120	Glu His Ile 125	Leu Ser Val
Lys Phe Val Gly 130	Gly Asp His 135	Arg Leu Arg	Asn Tyr Ser 140	Ser Ile Val
Thr Val His Pro 145	Glu Ser Ile 150	Asp Gly Arg	Pro Gly Thr 155	Leu Val Ile 160
Glu Ser Phe Val	Val Asp Val 165	Pro Asp Gly 170	Asn Thr Lys	Asp Glu Thr 175
Cys Tyr Phe Val	Glu Ala Val	Ile Lys Cys 185		Ser Leu Ala 190
Glu Val Ser Glu 195	Arg Leu Ala	Val Gln Ser 200	Pro Thr Ser 205	Pro Leu Glu
Gln				

Gln

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<210> SEQ ID NO 63
<211> LENGTH: 180
<212> TYPE: PRT
<213> ORGANISM: Oryza sativa
<220> FEATURE:
<223> OTHER INFORMATION: rice Japonica cultivar group, cultivar
     Nipponbare (GA3), clone OSJNBa0052K15 Bet v I allergen-like
<400> SEQUENCE: 63
Met Val Glu Met Asp Ala Gly Gly Arg Pro Glu Pro Ser Pro Pro Ser
Gly Gln Cys Ser Ser Ala Val Thr Met Arg Ile Asn Ala Pro Val His
Leu Val Trp Ser Ile Val Arg Arg Phe Glu Glu Pro His Ile Phe Gln
Pro Phe Val Arg Gly Cys Thr Met Arg Gly Ser Thr Ser Leu Ala Val
Gly Cys Val Arg Glu Val Asp Phe Lys Ser Gly Phe Pro Ala Lys Ser
Ser Val Glu Arg Leu Glu Ile Leu Asp Asp Lys Glu His Val Phe Gly
Val Arg Ile Ile Gly Gly Asp His Arg Leu Lys Asn Tyr Ser Ser Val
          100
Leu Thr Ala Lys Pro Glu Val Ile Asp Gly Glu Pro Ala Thr Leu Val
Ser Glu Ser Phe Val Val Asp Val Pro Glu Gly Asn Thr Ala Asp Glu
                     135
Thr Arg His Phe Val Glu Phe Leu Ile Arg Cys Asn Leu Arg Ser Leu
                 150
                                      155
Ala Met Val Ser Gln Arg Leu Leu Leu Ala Gln Gly Asp Leu Ala Glu
               165
                                  170
Pro Pro Ala Gln
           180
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<211> LENGTH: 176
<212> TYPE: PRT
<213> ORGANISM: Vitis vinifera
<220> FEATURE:
<223> OTHER INFORMATION: grapevine cultivar Pinot Noir, clone ENTAV 115
     hypothetical protein, locus VITISV_029498
<400> SEQUENCE: 64
Met Asn Gly Asn Gly Leu Ser Ser Met Glu Ser Glu Tyr Ile Arg Arg
His His Arg His Glu Pro Ala Glu Asn Gln Cys Ser Ser Ala Leu Val
Lys His Ile Lys Ala Pro Val Pro Leu Val Trp Ser Leu Val Arg Arg
                       40
Phe Asp Gln Pro Gln Lys Tyr Lys Pro Phe Ile Ser Arg Cys Val Val
Gln Gly Asn Leu Glu Ile Gly Ser Leu Arg Glu Val Asp Val Lys Ser
Gly Leu Pro Ala Thr Thr Ser Thr Glu Arg Leu Glu Leu Leu Asp Asp
                      90
Asp Glu His Ile Leu Ser Met Arg Ile Ile Gly Gly Asp His Arg Leu
                               105
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Arg Asn Tyr Ser Ser Ile Ile Ser Leu His Pro Glu Ile Ile Asp Gly
   115
                         120
Arg Pro Gly Thr Met Val Ile Glu Ser Tyr Val Val Asp Val Pro Glu
Gly Asn Thr Lys Asp Glu Thr Cys Tyr Phe Ser Leu Ala Asp Val Ser
     150
                          155
Glu Arg Leu Ala Val Ala Gly Thr Val Thr Glu Pro Ile Asp Arg Met
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<213 > ORGANISM: Oryza sativa
<220> FEATURE:
<223> OTHER INFORMATION: rice Indica cultivar group, cultivar 93-11
     hypothetical protein OsI_06615, Bet v I
     allergen-like protein
<400> SEQUENCE: 65
Met Val Glu Met Asp Ala Gly Gly Arg Pro Glu Pro Ser Pro Pro Ser
Gly Gln Cys Ser Ser Ala Val Thr Met Arg Ile Asn Ala Pro Val His
Leu Val Trp Ser Ile Val Arg Arg Phe Glu Glu Pro His Ile Phe Gln
                        40
Pro Phe Val Arg Gly Cys Thr Met Arg Gly Ser Thr Ser Leu Ala Val
                     55
Gly Cys Val Arg Glu Val Asp Phe Lys Ser Gly Phe Ser Ala Lys Ser
Ser Val Glu Arg Leu Glu Ile Leu Asp Asp Lys Glu His Val Phe Gly
Val Arg Ile Ile Gly Gly Asp His Arg Leu Lys Asn Tyr Ser Ser Val
                           105
         100
Leu Thr Ala Lys Pro Glu Val Ile Asp Gly Glu Pro Ala Thr Leu Val
                          120
Ser Glu Ser Phe Val Ile Asp Val Pro Glu Gly Asn Thr Ala Asp Glu
Thr Arg His Phe Val Glu Phe Leu Ile Arg Cys Asn Leu Arg Ser Leu
        150 155
Ala Met Val Ser Gln Arg Leu Leu Leu Ala Gln Gly Asp Leu Ala Glu
Pro Pro Ala Gln
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<220> FEATURE:
<223> OTHER INFORMATION: rice Japonica cultivar group, cultivar
     Nipponbare hypothetical protein OsJ_10498
<400> SEQUENCE: 66
Met Pro Cys Ile Pro Ala Ser Ser Pro Gly Ile Pro His Gln His Gln
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His Gln His His Arg Ala Leu Ala Gly Val Gly Met Ala Val Gly Cys
                             25
Ala Ala Glu Ala Ala Val Ala Ala Ala Gly Val Ala Gly Thr Arg Cys
                   40
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Gly Ala His Asp Gly Glu Val Pro Met Glu Val Ala Arg His His Glu His Ala Glu Pro Gly Ser Gly Arg Cys Cys Ser Ala Val Val Gln His 65  $\phantom{00}70\phantom{00}70\phantom{00}75\phantom{00}75\phantom{00}80\phantom{00}$ Val Ala Ala Pro Ala Ala Ala Val Trp Ser Val Val Arg Arg Phe Asp Gln Pro Gln Ala Tyr Lys Arg Phe Val Arg Ser Cys Ala Leu Leu Ala Gly Asp Gly Gly Leu Gly Lys Val Arg Glu Arg Leu Glu Ile Leu Asp Asp Glu Ser His Val Leu Ser Phe Arg Val Val Gly Gly Glu His Arg Leu Lys Asn Tyr Leu Ser Val Thr Thr Val His Pro Ser Pro Ser Ala Pro Thr Ala Ala Thr Val Val Val Glu Ser Tyr Val Val Asp Val Pro 165 170 Pro Gly Asn Thr Pro Glu Asp Thr Arg Val Phe Val Asp Thr Ile Val 185 Lys Cys Asn Leu Gln Ser Leu Ala Lys Thr Ala Glu Lys Leu Ala Ala 200 Gly Ala Arg Ala Ala Gly Ser 210 <210> SEQ ID NO 67 <211> LENGTH: 186 <212> TYPE: PRT <213> ORGANISM: Rheum australe <220> FEATURE: <223> OTHER INFORMATION: Himalayan rhubarb (Rheum emodi) pathogen-induced protein-like protein <400> SEQUENCE: 67 Met Asn Gly Asp Gly Tyr Gly Gly Ser Glu Glu Glu Phe Val Lys Arg Tyr His Glu His Val Leu Ala Asp His Gln Cys Ser Ser Val Leu Val Glu His Ile Asn Ala Pro Leu His Leu Val Trp Ser Leu Val Arg Ser Phe Asp Gln Pro Gln Lys Tyr Lys Pro Phe Val Ser Arg Cys Val Val Gln Gly Gly Asp Leu Glu Ile Gly Ser Val Arg Glu Val Asp Val Lys Ser Gly Leu Pro Ala Thr Thr Ser Met Glu Glu Leu Glu Leu Leu Asp Asp Lys Glu His Val Leu Arg Val Lys Phe Val Gly Gly Asp His Arg Leu Lys Asn Tyr Ser Ser Ile Val Ser Leu His Pro Glu Ile Ile Gly 120 Gly Arg Ser Gly Thr Met Val Ile Glu Ser Phe Ile Val Asp Ile Ala 135 Asp Gly Asn Thr Lys Glu Glu Thr Cys Tyr Phe Ile Glu Ser Leu Ile Asn Cys Asn Leu Lys Ser Leu Ser Cys Val Ser Glu Arg Leu Ala Val Glu Asp Ile Ala Glu Arg Ile Ala Gln Met

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185

180

<210> SEQ ID NO 68 <211> LENGTH: 254 <212> TYPE: PRT <213> ORGANISM: Oryza sativa <220> FEATURE: <223> OTHER INFORMATION: rice Japonica cultivar group, cultivar Nipponbare hypothetical protein OsJ\_016770 <400> SEQUENCE: 68 Met Val Gly Leu Val Gly Gly Gly Gly Trp Arg Val Gly Asp Asp Ala 1 5 10 15 Ala Gly Gly Gly Gly Gly Ala Val Ala Ala Gly Ala Ala Ala Ala Ala Glu Ala Glu His Met Arg Arg Leu His Ser Gln Gly Pro Arg Arg Ala Pro Val Gln Leu Arg Ala Arg Gln Ala His Gln Gly Ser Cys Ser Pro Pro Arg Ile Glu Cys Ala Asn Phe Ala Val Phe Leu Ala Ala Arg Asp Pro Lys Ile Val Trp Ser Leu Val Arg Ser Phe Asp Gln Pro Gln Arg Tyr Lys Pro Phe Val Ser Arg Cys Val Val Arg Gly Gly Asp Leu 105 Glu Ile Gly Ser Val Arg Glu Val Asn Val Lys Thr Gly Leu Pro Ala 120 Thr Thr Ser Thr Glu Arg Leu Glu Leu Leu Asp Asp Asp Glu His Ile 135 Leu Ser Val Lys Phe Val Gly Gly Asp His Arg Leu Arg Asn Tyr Ser Ser Ile Val Thr Val His Pro Glu Ser Ile Asp Gly Arg Pro Gly Thr 170 Leu Val Ile Glu Ser Phe Val Val Asp Val Pro Asp Gly Asn Thr Lys Asp Glu Thr Cys Tyr Phe Val Glu Ala Val Ile Lys Cys Asn Leu Thr 200 Ser Leu Ala Glu Met Val Arg Met Ile Ser Leu Val Leu Pro Phe Met Leu Val Asp Arg Met Ser Gly Ile Thr Cys Glu Ser His Leu Glu Thr 235 Thr Leu Val Arg Cys Gly Glu Tyr Ala Val Leu Ala His Val <210> SEQ ID NO 69 <211> LENGTH: 186 <212> TYPE: PRT <213> ORGANISM: Oryza sativa <220> FEATURE: <223> OTHER INFORMATION: rice Japonica cultivar group, cultivar Nipponbare hypothetical protein OsJ\_005784 <400> SEQUENCE: 69 Met Glu Pro His Met Glu Arg Ala Leu Arg Glu Ala Val Ala Ser Glu Ala Glu Arg Arg Glu Leu Glu Gly Val Val Arg Ala His His Thr Gly

Trp Asn Ala Pro Leu Ala Ala Val Trp Pro His Arg Ala Arg Val Arg

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		35					40					45			
Pro	Thr 50	Arg	Ser	Gly	Thr	Ser 55	Thr	Ser	Ser	Ser	Arg 60	Ala	Ser	Ser	Pro
Pro 65	Gly	Asp	Gly	Ala	Thr 70	Val	Gly	Ser	Val	Arg 75	Glu	Val	Ala	Val	Val 80
Ser	Gly	Leu	Pro	Ala 85	Ser	Thr	Ser	Thr	Glu 90	Arg	Leu	Glu	Ile	Leu 95	Asp
Asp	Asp	Arg	His 100	Val	Leu	Ser	Phe	Arg 105	Val	Val	Gly	Gly	Asp 110	His	Arg
Leu	Arg	Asn 115	Tyr	Arg	Ser	Val	Thr 120	Ser	Val	Thr	Glu	Phe 125	Ser	Ser	Pro
Ser	Ser 130	Pro	Pro	Arg	Pro	Tyr 135	Cys	Val	Val	Val	Glu 140	Ser	Tyr	Val	Val
Asp 145	Val	Pro	Glu	Gly	Asn 150	Thr	Glu	Glu	Asp	Thr 155	Arg	Met	Phe	Thr	Asp 160
Thr	Val	Val	Lys	Leu 165	Asn	Leu	Gln	Lys	Leu 170	Ala	Ala	Val	Ala	Thr 175	Ser
Ser	Ser	Pro	Pro 180	Ala	Ala	Gly	Asn	His 185	His						
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< 400	)> SI	EQUEI	ICE :	70											
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Phe	Gln	Pro	Phe 20	Val	Arg	Gly	СЛа	Thr 25	Met	Arg	Gly	Ser	Thr 30	Ser	Leu
Ala	Val	Gly 35	Сув	Val	Arg	Glu	Val 40	Asp	Phe	Lys	Ser	Gly 45	Phe	Pro	Ala
Lys	Ser 50	Ser	Val	Glu	Arg	Leu 55	Glu	Ile	Leu	Asp	Asp 60	Lys	Glu	His	Val
Phe 65	Gly	Val	Arg	Ile	Ile 70	Gly	Gly	Asp	His	Arg 75	Leu	Lys	Asn	Tyr	Ser 80
Ser	Val	Leu	Thr	Ala 85	ГЛа	Pro	Glu	Val	Ile 90	Asp	Gly	Glu	Pro	Ala 95	Thr
Leu	Val	Ser	Glu 100	Ser	Phe	Val	Val	Asp 105	Val	Pro	Glu	Gly	Asn 110	Thr	Ala
Asp	Glu	Thr 115	Arg	His	Phe	Val	Glu 120	Phe	Leu	Ile	Arg	Сув 125	Asn	Leu	Arg
Ser	Leu 130	Ala	Met	Val	Ser	Gln 135	Arg	Leu	Leu	Leu	Ala 140	Gln	Gly	Asp	Leu
Ala 145	Glu	Pro	Pro	Gly	Gln 150										
<213 <213 <213 <220		ENGTI PE: RGAN EATUI PHER	H: 20 PRT ISM: RE: INFO	Of Ory: ORMA:	CION	: rio		-				-	ль' (	culta	ivar

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<400> SEQUENCE: 71 Met Pro Tyr Thr Ala Pro Arg Pro Ser Pro Pro Gln His Ser Arg Ile 10 Gly Gly Cys Gly Gly Gly Val Leu Lys Ala Ala Gly Ala Ala Gly His Ala Ala Ser Cys Val Ala Val Pro Ala Glu Val Ala Arg His His Glu His Ala Ala Gly Val Gly Gln Cys Cys Ser Ala Val Val Gln Ala Ile Ala Ala Pro Val Asp Ala Val Trp Arg Thr Ser Thr Ser Ser Gly Ala Ala Ala Ser Trp Thr Ala Thr Ala Thr Ala Gly Pro Leu Pro Val Gly Ser Val Arg Glu Phe Arg Val Leu Ser Gly Leu Pro Gly Thr Ser Ser Arg Glu Arg Leu Glu Ile Leu Asp Asp Glu Arg Arg Val Leu Ser 120 Phe Arg Val Val Gly Glu His Arg Leu Ser Asn Tyr Arg Ser Val 135 Thr Thr Val His Glu Thr Ala Ala Gly Ala Ala Ala Ala Val Val 150 155 Glu Ser Tyr Val Val Asp Val Pro His Gly Asn Thr Ala Asp Glu Thr Arg Met Phe Val Asp Thr Ile Val Arg Cys Asn Leu Gln Ser Leu Ala 185 Arg Thr Ala Glu Gln Leu Ala Leu Ala Ala Pro Arg Ala Ala 200 <210> SEQ ID NO 72 <211> LENGTH: 396 <212> TYPE: PRT <213> ORGANISM: Vitis vinifera <220> FEATURE: <223> OTHER INFORMATION: grapevine cultivar Pinot Noir, clone ENTAV 115 hypothetical protein, locus VITISV\_001710 <221> NAME/KEY: VARIANT <222> LOCATION: (1)...(395) <223> OTHER INFORMATION: Xaa = any amino acid <400> SEQUENCE: 72 Met Pro Ile Ser Ser Leu Pro Phe Ser Leu Tyr Thr Val Thr Pro Asn Pro Leu Lys Leu Ile Thr Thr His Ala His Ala Phe Thr Pro His Thr His Ile Phe Thr Leu Lys Phe Met Ser His Thr Tyr Cys Pro His Ile  $35 \hspace{1.5cm} 40 \hspace{1.5cm} 45$ His His Ile Thr Ser Ile His Tyr Thr His Leu Leu Xaa Pro Ile Pro 55 His Met Pro Leu Gln Pro Pro Leu Pro Pro His Pro Ile Leu Pro Ser Met Pro Ala Phe Gln His Leu Tyr Ser Thr Asn Gln His Leu Gln Val Ala Leu Phe Ser Ala Arg Gly Pro Asn Ile Arg Asp Phe Asn Phe Gln 105 Asp Ala Asp Leu Leu Lys Leu Asp Ile Leu Ala Pro Gly Ser Leu Ile 120

Trp	Ala 130	Ala	Trp	Ser	Pro	Asn 135	Gly	Thr	Asp	Glu	Ala 140	Asn	Tyr	Val	Gly
Glu 145	Gly	Ser	Pro	Thr	Val 150	Ala	Met	Ile	Ala	Lys 155	Arg	Gly	Pro	Arg	His 160
Gly	Lys	Tyr	Met	Ala 165	Phe	Cys	Xaa	Met	Tyr 170	Arg	Asp	Asn	Val	Ala 175	Pro
ГЛа	Gly	Val	Asn 180	Xaa	Ala	Val	Ala	Thr 185	Val	Lys	Thr	ГÀа	Arg 190	Thr	Ile
Gln	Leu	Lys 195	Thr	Ser	Leu	Glu	Ile 200	Ala	СЛа	His	Tyr	Ala 205	Gly	Ile	Asn
Ile	Ser 210	Gly	Ile	Asn	Gly	Glu 215	Val	Met	Pro	Gly	Gln 220	Trp	Glu	Tyr	Gln
Val 225	Gly	Pro	Gly	Gln	Cys 230	Ser	Ser	Leu	Leu	Ala 235	Gln	Arg	Val	His	Val 240
Pro	Leu	Ser	Ala	Val 245	Gly	Ser	Val	Val	His 250	Arg	Phe	Asp	Lys	Pro 255	Gln
Arg	Tyr	Gln	His 260	Val	Ile	Lys	Ser	Сув 265	Arg	Ile	Glu	Asp	Gly 270	Phe	Glu
Met	Arg	Met 275	Gly	Xaa	Leu	Arg	Asp 280	Val	Asn	Ile	Ile	Ser 285	Gly	Leu	Pro
Thr	Ala 290	Thr	Asn	Thr	Gly	Arg 295	Leu	Asp	Met	Gln	Asp	Asp	Glu	Arg	His
Val 305	Thr	Arg	СЛа	Pro	His 310	Gln	Arg	Gln	Ser	Glu 315	Ser	ГÀа	Tyr	Thr	Glu 320
Asn	Asn	Asn	Ser	Asp 325	Ala	Ser	Ser	Ile	330 Lys	Ser	Pro	Ile	Asn	Gly 335	Pro
Ser	Glu	His	Leu 340	Lys	Thr	Ala	Ala	Ser 345	Pro	Lys	Thr	Glu	Ser 350	Ile	Ile
Val	Ile	Asp 355	Thr	Ser	Lys	Phe	Leu 360	Asn	Glu	Glu	Asp	Phe 365	Glu	Gly	Lys
Asp	Glu 370	Thr	Ser	Ser	Ser	Asn 375	Gln	Val	Gln	Ile	Glu 380	Asp	Glu	Asn	Trp
Glu 385	Thr	Arg	Phe	Pro	Asn 390	Thr	Asp	Ala	Gly	Ile 395	Trp				
<213 <213 <213 <223 <223 <223	0 > FI 3 > O' hy 1 > NA 2 > LO	ENGTI YPE: RGAN: EATUI THER YPOtl AME/I	H: 44 PRT ISM: RE: INFO netic KEY: ION:	Vit: DRMA Cal j VAR: (1)	prot	: gra ∋in,	apev: loci	ıs V:	ITIS7	J_014	1403	ot No	oir,	clo	ne ENTAV 115
< 400	O> SI	EQUEI	ICE :	73											
Met 1	Pro	Ser	Ala	Xaa 5	ГÀа	Ser	Ser	Thr	Val 10	Pro	Leu	Ser	Leu	Xaa 15	Gln
Phe	ГЛа	Leu	Gly 20	Leu	Arg	His	Gly	His 25	Arg	Val	Ile	Pro	Trp 30	Gly	Asp
Leu	Asp	Ser 35	Leu	Ala	Met	Leu	Gln 40	Arg	Gln	Leu	Asp	Val 45	Asp	Ile	Leu
Val	Thr 50	Gly	His	Thr	His	Arg 55	Phe	Thr	Ala	Tyr	Fys	His	Glu	Gly	Gly
Val 65	Val	Ile	Asn	Pro	Gly 70	Ser	Ala	Thr	Gly	Ala 75	Phe	Gly	Ser	Ile	Thr 80

Tyr	Asp	Val	Asn	Pro 85	Ser	Phe	Val	Leu	Met 90	Asp	Ile	Asp	Gly	Leu 95	Arg
Val	Val	Val	Cys	Val	Tyr	Glu	Leu	Ile 105	Asp	Glu	Thr	Ala	Asn 110	Ile	Ile
Lys	Glu	Leu 115	His	Ala	Arg	Lys	Ile 120	Ser	Phe	Gly	Thr	Lys 125	Ser	Met	Ile
Xaa	Cys 130	Leu	Leu	Leu	Lys	Arg 135	Arg	Ser	Thr	Pro	Lys 140	Phe	Arg	Arg	ГЛа
Lys 145	Leu	Phe	Leu	Phe	Gln 150	Cys	Arg	Val	Gln	Met 155	Thr	Leu	Thr	Leu	Thr 160
Asn	Leu	Ala	Val	Ser 165	Gly	Ile	Ala	Gln	Thr 170	Leu	Gln	Val	Asp	Gln 175	Trp
Thr	Val	Сув	Ala 180	Leu	Ile	Phe	Met	Thr 185	Arg	Arg	Asp	Ile	His 190	Leu	Asp
Lys	Ala	Arg 195	Phe	Leu	Asp	Phe	Lys 200	Asp	Met	Gly	Lys	Leu 205	Leu	Ala	Asp
Ala	Ser 210	Gly	Leu	Arg	Lys	Ala 215	Leu	Ser	Gly	Gly	Xaa 220	Val	Thr	Ala	Gly
Met 225	Ala	Ile	Phe	Asp	Thr 230	Met	Arg	His	Ile	Arg 235	Pro	Asp	Val	Pro	Thr 240
Val	Cys	Val	Gly	Leu 245	Ala	Ala	Val	Ala	Met 250	Ile	Ala	Lys	Arg	Gly 255	Pro
Arg	His	Gly	Lys 260	Tyr	Met	Ala	Phe	Сув 265	Pro	Met	Tyr	Arg	Asp 270	Asn	Val
Ala	Pro	Lys 275	Gly	Val	Asn	Val	Ala 280	Val	Val	Thr	Val	Lys 285	Thr	Lys	Arg
Thr	Ile 290	Gln	Leu	ГÀа	Thr	Ser 295	Leu	Glu	Ile	Ala	300 GÀa	His	Tyr	Ala	Gly
Ile 305	Asn	Ile	Ser	Gly	Ile 310	Asn	Gly	Glu	Val	Met 315	Pro	Gly	Gln	Trp	Glu 320
Tyr	Gln	Val	Gly	Pro 325	Gly	Gln	CÀa	Ser	Ser 330	Leu	Leu	Ala	Gln	Arg 335	Val
His	Val	Pro	Leu 340	Ser	Ala	Val	Gly	Ser 345	Val	Val	His	Arg	Phe 350	Asp	TÀa
Pro	Gln	Arg 355	Tyr	Gln	His	Val	Ile 360	Lys	Ser	Cys	Arg	Ile 365	Glu	Asp	Gly
Phe	Glu 370	Met	Arg	Met	Gly	Arg 375	Leu	Arg	Asp	Val	Asn 380	Ile	Ile	Ser	Gly
Leu 385	Pro	Thr	Ala	Thr	Asn 390	Thr	Gly	Arg	Leu	Asp 395	Met	Gln	Asp	Asp	Glu 400
Xaa	His	Val	Thr	Arg 405	CÀa	Pro	His	Gln	Arg 410	Gln	Ser	Glu	Ser	Lys 415	Tyr
Thr	Glu	Asn	Asn 420	Asn	Ser	Asp	Ala	Ser 425	Ser	Val	ГЛа	Ser	Pro 430	Ile	Asn
Gly	Pro	Ser 435	Glu	His	Leu	Lys	Thr 440	Ala	Ala	Xaa					
<213 <213 <213 <220	L> LI 2> T: 3> OI 0> FI 3> O:	EATUI CHER	H: 9! PRT ISM: RE: INFO	ory:	rion	: ri	ce In						, cul	ltiva	ar Pokkali,

clone OSR-385-428-D5 capip1 protein partial sequence

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Glu Ile Gly Ser Val Arg Glu Val Asn Val Lys Thr Gly Leu Pro Ala
Thr Thr Ser Thr Glu Arg Leu Glu Leu Leu Asp Asp Asp Glu His Ile
Leu Ser Val Lys Phe Val Gly Gly Asp His Arg Leu Arg Asn Tyr Ser
Ser Ile Val Thr Val His Pro Glu Ser Ile Asp Gly Arg Pro Gly Thr
Leu Val Ile Glu Ser Phe Val Val Asp Val Pro Asp Gly Asn Thr Lys
Asp Glu Thr Cys Tyr Phe Val Glu Ala Val Ile Lys Cys Asn Leu
<210> SEQ ID NO 75
<211> LENGTH: 191
<212> TYPE: PRT
<213 > ORGANISM: Zea mays
<220> FEATURE:
<223 > OTHER INFORMATION: corn (maize) strain B73, clone ZM_BFc0034007
     unknown protein
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Gly Gly Ala Gln Thr Pro Ala Pro Pro Pro Pro Arg Arg Trp Arg Leu
Ala Asp Glu Arg Cys Asp Leu Arg Ala Met Glu Thr Asp Tyr Val Arg
                 40
Arg Phe His Arg His Glu Pro Arg Asp His Gln Cys Ser Ser Ala Val
                      55
Ala Lys His Ile Lys Ala Pro Val His Leu Val Trp Ser Leu Val Arg
Arg Phe Asp Gln Pro Gln Leu Phe Lys Pro Phe Val Ser Arg Cys Glu
Met Lys Gly Asn Ile Glu Ile Gly Ser Val Arg Glu Val Asn Val Lys
Ser Gly Leu Pro Ala Thr Arg Ser Thr Glu Arg Leu Glu Leu Leu Asp
Asp Asp Glu Arg Ile Leu Ser Val Arg Phe Val Gly Gly Asp His Arg
Leu Gln Val Cys Ser Val Leu His Leu Ser Ile Phe Cys Ala Ala His
Ala Arg Tyr Phe Ala His His Leu Lys Cys Val Leu Glu Phe Leu Cys
Gln Met His Leu Asp Val Leu Pro Cys Asp Asp Ala Ile Leu Glu
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<210> SEQ ID NO 76
<211 > LENGTH: 239
<212> TYPE: PRT
<213> ORGANISM: Oryza sativa
<220> FEATURE:
<223> OTHER INFORMATION: rice Japonica cultivar group, cultivar
     Nipponbare hypothetical protein OsJ_020681
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<400> SEQUENCE: 76

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Thr Glu Glu Glu Thr Thr Leu Phe Ala Asp Thr Ile Val Gly Cys Asn 130 135 Leu Arg Ser Leu Ala Lys Leu Ser Glu Lys Met Met Glu Leu Thr <210> SEQ ID NO 78 <211> LENGTH: 188 <212> TYPE: PRT <213> ORGANISM: Arabidopsis sp. <220> FEATURE: <223> OTHER INFORMATION: PYR/PYL receptor polypeptide PYL8 <400> SEQUENCE: 78 Met Glu Ala Asn Gly Ile Glu Asn Leu Thr Asn Pro Asn Gln Glu Arg Glu Phe Ile Arg Arg His His Lys His Glu Leu Val Asp Asn Gln Cys Ser Ser Thr Leu Val Lys His Ile Asn Ala Pro Val His Ile Val Trp 40 Ser Leu Val Arg Arg Phe Asp Gln Pro Gln Lys Tyr Lys Pro Phe Ile 55 Ser Arg Cys Val Val Lys Gly Asn Met Glu Ile Gly Thr Val Arg Glu 65 70 75 80 Val Asp Val Lys Ser Gly Leu Pro Ala Thr Arg Ser Thr Glu Arg Leu Glu Leu Leu Asp Asp Asn Glu His Ile Leu Ser Ile Arg Ile Val Gly 105 Gly Asp His Arg Leu Lys Asn Tyr Ser Ser Ile Ile Ser Leu His Pro 120 Glu Thr Ile Glu Gly Arg Ile Gly Thr Leu Val Ile Glu Ser Phe Val Val Asp Val Pro Glu Gly Asn Thr Lys Asp Glu Thr Cys Tyr Phe Val 150 155 Glu Ala Leu Ile Lys Cys Asn Leu Lys Ser Leu Ala Asp Ile Ser Glu Arg Leu Ala Val Gln Asp Thr Thr Glu Ser Arg Val 180 <210> SEQ ID NO 79 <211> LENGTH: 211 <212> TYPE: PRT <213 > ORGANISM: Arabidopsis sp. <220> FEATURE: <223> OTHER INFORMATION: PYR/PYL receptor polypeptide PYL7 <400> SEQUENCE: 79 Met Glu Met Ile Gly Gly Asp Asp Thr Asp Thr Glu Met Tyr Gly Ala Leu Val Thr Ala Gln Ser Leu Arg Leu Arg His Leu His His Cys Arg 25 Glu Asn Gln Cys Thr Ser Val Leu Val Lys Tyr Ile Gln Ala Pro Val 40 His Leu Val Trp Ser Leu Val Arg Arg Phe Asp Gln Pro Gln Lys Tyr 55 Lys Pro Phe Ile Ser Arg Cys Thr Val Asn Gly Asp Pro Glu Ile Gly 70 75 Cys Leu Arg Glu Val Asn Val Lys Ser Gly Leu Pro Ala Thr Thr Ser 90

Thr Glu Arg Leu Glu Gln Leu Asp Asp Glu Glu His Ile Leu Gly Ile Asn Ile Ile Gly Gly Asp His Arg Leu Lys Asn Tyr Ser Ser Ile Leu Thr Val His Pro Glu Met Ile Asp Gly Arg Ser Gly Thr Met Val Met Glu Ser Phe Val Val Asp Val Pro Gln Gly Asn Thr Lys Asp Asp Thr Cys Tyr Phe Val Glu Ser Leu Ile Lys Cys Asn Leu Lys Ser Leu Ala Cys Val Ser Glu Arg Leu Ala Ala Gln Asp Ile Thr Asn Ser Ile Ala Thr Phe Cys Asn Ala Ser Asn Gly Tyr Arg Glu Lys Asn His Thr Glu Thr Asn Leu 210 <210> SEO ID NO 80 <211> LENGTH: 187 <212> TYPE: PRT <213> ORGANISM: Arabidopsis sp. <220> FEATURE: <223> OTHER INFORMATION: PYR/PYL receptor polypeptide PYL9 <400> SEQUENCE: 80 Met Met Asp Gly Val Glu Gly Gly Thr Ala Met Tyr Gly Gly Leu Glu Thr Val Gln Tyr Val Arg Thr His His Gln His Leu Cys Arg Glu Asn Gln Cys Thr Ser Ala Leu Val Lys His Ile Lys Ala Pro Leu His Leu Val Trp Ser Leu Val Arg Arg Phe Asp Gln Pro Gln Lys Tyr Lys Pro Phe Val Ser Arg Cys Thr Val Ile Gly Asp Pro Glu Ile Gly Ser Leu Arg Glu Val Asn Val Lys Ser Gly Leu Pro Ala Thr Thr Ser Thr Glu Arg Leu Glu Leu Leu Asp Asp Glu Glu His Ile Leu Gly Ile Lys Ile Ile Gly Gly Asp His Arg Leu Lys Asn Tyr Ser Ser Ile Leu Thr Val His Pro Glu Ile Ile Glu Gly Arg Ala Gly Thr Met Val Ile Glu Ser Phe Val Val Asp Val Pro Gln Gly Asn Thr Lys Asp Glu Thr Cys Tyr 155 Phe Val Glu Ala Leu Ile Arg Cys Asn Leu Lys Ser Leu Ala Asp Val 170 Ser Glu Arg Leu Ala Ser Gln Asp Ile Thr Gln 180 <210> SEQ ID NO 81 <211> LENGTH: 161 <212> TYPE: PRT <213> ORGANISM: Arabidopsis sp. <220> FEATURE: <223> OTHER INFORMATION: PYR/PYL receptor polypeptide PYL11

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Met Glu Thr Ser Gln Lys Tyr His Thr Cys Gly Ser Thr Leu Val Gln
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Thr Ile Asp Ala Pro Leu Ser Leu Val Trp Ser Ile Leu Arg Arg Phe
Asp Asn Pro Gln Ala Tyr Lys Gln Phe Val Lys Thr Cys Asn Leu Ser
Ser Gly Asp Gly Gly Glu Gly Ser Val Arg Glu Val Thr Val Val Ser
Gly Leu Pro Ala Glu Phe Ser Arg Glu Arg Leu Asp Glu Leu Asp Asp
Glu Ser His Val Met Met Ile Ser Ile Ile Gly Gly Asp His Arg Leu
Val Asn Tyr Arg Ser Lys Thr Met Ala Phe Val Ala Ala Asp Thr Glu
Glu Lys Thr Val Val Val Glu Ser Tyr Val Val Asp Val Pro Glu Gly
                         120
Asn Ser Glu Glu Glu Thr Thr Ser Phe Ala Asp Thr Ile Val Gly Phe
                     135
Asn Leu Lys Ser Leu Ala Lys Leu Ser Glu Arg Val Ala His Leu Lys
                 150
                                     155
Leu
<210> SEQ ID NO 82
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<213> ORGANISM: Arabidopsis sp.
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His His Arg His Glu Leu Val Glu Ser Gln Cys Ser Ser Thr Leu Val
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Lys His Ile Lys Ala Pro Leu His Leu Val Trp Ser Ile Val Arg Arg
Phe Asp Glu Pro Gln Lys Tyr Lys Pro Phe Ile Ser Arg Cys Val Val
Gln Gly Lys Lys Leu Glu Val Gly Ser Val Arg Glu Val Asp Leu Lys
Ser Gly Leu Pro Ala Thr Lys Ser Thr Glu Val Leu Glu Ile Leu Asp
Asp Asn Glu His Ile Leu Gly Ile Arg Ile Val Gly Gly Asp His Arg
Leu Lys Asn Tyr Ser Ser Thr Ile Ser Leu His Ser Glu Thr Ile Asp
                         120
Gly Lys Thr Gly Thr Leu Ala Ile Glu Ser Phe Val Val Asp Val Pro
           135
Glu Gly Asn Thr Lys Glu Glu Thr Cys Phe Phe Val Glu Ala Leu Ile
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Gln Cys Asn Leu Asn Ser Leu Ala Asp Val Thr Glu Arg Leu Gln Ala
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                                  170
Glu Ser Met Glu Lys Lys Ile
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<210> SEQ ID NO 83
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<212> TYPE: PRT
<213> ORGANISM: Arabidopsis sp.
<220> FEATURE:
<223> OTHER INFORMATION: PYR/PYL receptor polypeptide PYL13
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Ile Glu Ala Pro Leu Pro Leu Val Trp Ser Ile Leu Arg Ser Phe Asp
Lys Pro Gln Ala Tyr Gln Arg Phe Val Lys Ser Cys Thr Met Arg Ser
Gly Gly Gly Gly Gly Lys Gly Glu Gly Lys Gly Ser Val Arg Asp 50 \\ 60
Val Thr Leu Val Ser Gly Phe Pro Ala Asp Phe Ser Thr Glu Arg Leu 65 70 75 80
Glu Glu Leu Asp Asp Glu Ser His Val Met Val Val Ser Ile Ile Gly
Gly Asn His Arg Leu Val Asn Tyr Lys Ser Lys Thr Lys Val Val Ala
          100
                               105
Ser Pro Glu Asp Met Ala Lys
       115
<210> SEO ID NO 84
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<213> ORGANISM: Arabidopsis sp.
<220> FEATURE:
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Phe His Thr Leu Gln Pro His Asp Gln Thr Asp Gly Pro Ile Lys Arg
Val Cys Leu Thr Arg Gly Met His Val Pro Glu His Val Ala Met His
                 40
His Thr His Asp Val Gly Pro Asp Gln Cys Cys Ser Ser Val Val Gln
Met Ile His Ala Pro Pro Glu Ser Val Trp Ala Leu Val Arg Arg Phe 65 70 75 80
Asp Asn Pro Lys Val Tyr Lys Asn Phe Ile Arg Gln Cys Arg Ile Val
Gln Gly Asp Gly Leu His Val Gly Asp Leu Arg Glu Val Met Val Val
Ser Gly Leu Pro Ala Val Ser Ser Thr Glu Arg Leu Glu Ile Leu Asp
                           120
Glu Glu Arg His Val Ile Ser Phe Ser Val Val Gly Gly Asp His Arg
                       135
Leu Lys Asn Tyr Arg Ser Val Thr Thr Leu His Ala Ser Asp Asp Glu
                                      155
Gly Thr Val Val Val Glu Ser Tyr Ile Val Asp Val Pro Pro Gly Asn
Thr Glu Glu Glu Thr Leu Ser Phe Val Asp Thr Ile Val Arg Cys Asn
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185 190 Leu Gln Ser Leu Ala Arg Ser Thr Asn Arg Gln 195 <210> SEQ ID NO 85 <211> LENGTH: 207 <212> TYPE: PRT <213 > ORGANISM: Arabidopsis sp. <220> FEATURE: <223> OTHER INFORMATION: PYR/PYL receptor polypeptide PYL4 <400> SEQUENCE: 85 Met Leu Ala Val His Arg Pro Ser Ser Ala Val Ser Asp Gly Asp Ser Val Gln Ile Pro Met Met Ile Ala Ser Phe Gln Lys Arg Phe Pro Ser Leu Ser Arg Asp Ser Thr Ala Ala Arg Phe His Thr His Glu Val Gly Pro Asn Gln Cys Cys Ser Ala Val Ile Gln Glu Ile Ser Ala Pro Ile Ser Thr Val Trp Ser Val Val Arg Arg Phe Asp Asn Pro Gln Ala Tyr Lys His Phe Leu Lys Ser Cys Ser Val Ile Gly Gly Asp Gly Asp Asn Val Gly Ser Leu Arg Gln Val His Val Val Ser Gly Leu Pro Ala Ala 100 105 Ser Ser Thr Glu Arg Leu Asp Ile Leu Asp Asp Glu Arg His Val Ile Ser Phe Ser Val Val Gly Gly Asp His Arg Leu Ser Asn Tyr Arg Ser 135 Val Thr Thr Leu His Pro Ser Pro Ile Ser Gly Thr Val Val Val Glu 150 155 Ser Tyr Val Val Asp Val Pro Pro Gly Asn Thr Lys Glu Glu Thr Cys Asp Phe Val Asp Val Ile Val Arg Cys Asn Leu Gln Ser Leu Ala Lys Ile Ala Glu Asn Thr Ala Ala Glu Ser Lys Lys Lys Met Ser Leu 200 <210> SEQ ID NO 86 <211> LENGTH: 215 <212> TYPE: PRT <213 > ORGANISM: Arabidopsis sp. <220> FEATURE: <223> OTHER INFORMATION: PYR/PYL receptor polypeptide PYL6 <400> SEQUENCE: 86 Met Pro Thr Ser Ile Gln Phe Gln Arg Ser Ser Thr Ala Ala Glu Ala 10 Ala Asn Ala Thr Val Arg Asn Tyr Pro His His His Gln Lys Gln Val Gln Lys Val Ser Leu Thr Arg Gly Met Ala Asp Val Pro Glu His Val 40 Glu Leu Ser His Thr His Val Val Gly Pro Ser Gln Cys Phe Ser Val Val Val Gln Asp Val Glu Ala Pro Val Ser Thr Val Trp Ser Ile Leu

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Ser Arg Phe Glu His Pro Gln Ala Tyr Lys His Phe Val Lys Ser Cys
His Val Val Ile Gly Asp Gly Arg Glu Val Gly Ser Val Arg Glu Val
Arg Val Val Ser Gly Leu Pro Ala Ala Phe Ser Leu Glu Arg Leu Glu
                120
Ile Met Asp Asp Asp Arg His Val Ile Ser Phe Ser Val Val Gly Gly
Asp His Arg Leu Met Asn Tyr Lys Ser Val Thr Thr Val His Glu Ser
Glu Glu Asp Ser Asp Gly Lys Lys Arg Thr Arg Val Val Glu Ser Tyr
Val Val Asp Val Pro Ala Gly Asn Asp Lys Glu Glu Thr Cys Ser Phe
Ala Asp Thr Ile Val Arg Cys Asn Leu Gln Ser Leu Ala Lys Leu Ala
Glu Asn Thr Ser Lys Phe Ser
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<210> SEO ID NO 87
<211> LENGTH: 190
<212> TYPE: PRT
<213 > ORGANISM: Arabidopsis sp.
<220> FEATURE:
<223> OTHER INFORMATION: PYR/PYL receptor polypeptide PYL2
<400> SEQUENCE: 87
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Thr Leu Glu Pro Val Ile Lys Thr Tyr His Gln Phe Glu Pro Asp Pro
Thr Thr Cys Thr Ser Leu Ile Thr Gln Arg Ile His Ala Pro Ala Ser
Val Val Trp Pro Leu Ile Arg Arg Phe Asp Asn Pro Glu Arg Tyr Lys
His Phe Val Lys Arg Cys Arg Leu Ile Ser Gly Asp Gly Asp Val Gly
Ser Val Arg Glu Val Thr Val Ile Ser Gly Leu Pro Ala Ser Thr Ser
Thr Glu Arg Leu Glu Phe Val Asp Asp Asp His Arg Val Leu Ser Phe
                               105
Arg Val Val Gly Gly Glu His Arg Leu Lys Asn Tyr Lys Ser Val Thr
Ser Val Asn Glu Phe Leu Asn Gln Asp Ser Gly Lys Val Tyr Thr Val 130 135 140
Val Leu Glu Ser Tyr Thr Val Asp Ile Pro Glu Gly Asn Thr Glu Glu
                               155
Asp Thr Lys Met Phe Val Asp Thr Val Val Lys Leu Asn Leu Gln Lys
Leu Gly Val Ala Ala Thr Ser Ala Pro Met His Asp Asp Glu
<210> SEQ ID NO 88
<211> LENGTH: 209
<212> TYPE: PRT
<213 > ORGANISM: Arabidopsis sp.
<220> FEATURE:
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<223> OTHER INFORMATION: PYR/PYL receptor polypeptide PYL3 <400> SEQUENCE: 88 Met Asn Leu Ala Pro Ile His Asp Pro Ser Ser Ser Ser Thr Thr Thr Thr Ser Ser Ser Thr Pro Tyr Gly Leu Thr Lys Asp Glu Phe Ser Thr Leu Asp Ser Ile Ile Arg Thr His His Thr Phe Pro Arg Ser Pro Asn Thr Cys Thr Ser Leu Ile Ala His Arg Val Asp Ala Pro Ala His Ala Ile Trp Arg Phe Val Arg Asp Phe Ala Asn Pro Asn Lys Tyr Lys His Phe Ile Lys Ser Cys Thr Ile Arg Val Asn Gly Asn Gly Ile Lys Glu Ile Lys Val Gly Thr Ile Arg Glu Val Ser Val Val Ser Gly Leu Pro 105 Ala Ser Thr Ser Val Glu Ile Leu Glu Val Leu Asp Glu Glu Lys Arg 120 Ile Leu Ser Phe Arg Val Leu Gly Gly Glu His Arg Leu Asn Asn Tyr 135 Arg Ser Val Thr Ser Val Asn Glu Phe Val Val Leu Glu Lys Asp Lys Lys Lys Arg Val Tyr Ser Val Val Leu Glu Ser Tyr Ile Val Asp Ile 170 Pro Gln Gly Asn Thr Glu Glu Asp Thr Arg Met Phe Val Asp Thr Val 185 Val Lys Ser Asn Leu Gln Asn Leu Ala Val Ile Ser Thr Ala Ser Pro 200 Thr <210> SEQ ID NO 89 <211> LENGTH: 191 <212> TYPE: PRT <213> ORGANISM: Arabidopsis sp. <220> FEATURE: <223> OTHER INFORMATION: PYR/PYL receptor polypeptide PYR1 <400> SEQUENCE: 89 Met Pro Ser Glu Leu Thr Pro Glu Glu Arg Ser Glu Leu Lys Asn Ser Ile Ala Glu Phe His Thr Tyr Gln Leu Asp Pro Gly Ser Cys Ser Ser Leu His Ala Gln Arg Ile His Ala Pro Pro Glu Leu Val Trp Ser Ile Val Arg Arg Phe Asp Lys Pro Gln Thr Tyr Lys His Phe Ile Lys Ser Cys Ser Val Glu Gln Asn Phe Glu Met Arg Val Gly Cys Thr Arg Asp Val Ile Val Ile Ser Gly Leu Pro Ala Asn Thr Ser Thr Glu Arg Leu Asp Ile Leu Asp Asp Glu Arg Arg Val Thr Gly Phe Ser Ile Ile Gly 105 Gly Glu His Arg Leu Thr Asn Tyr Lys Ser Val Thr Thr Val His Arg

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Phe Glu Lys Glu Asn Arg Ile Trp Thr Val Val Leu Glu Ser Tyr Val
   130
                       135
Val Asp Met Pro Glu Gly Asn Ser Glu Asp Asp Thr Arg Met Phe Ala
Asp Thr Val Val Lys Leu Asn Leu Gln Lys Leu Ala Thr Val Ala Glu
                       170
Ala Met Ala Arg Asn Ser Gly Asp Gly Ser Gly Ser Gln Val Thr
<210> SEQ ID NO 90
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<212> TYPE: PRT
<213 > ORGANISM: Arabidopsis sp.
<220> FEATURE:
<223> OTHER INFORMATION: PYR/PYL receptor polypeptide PYL1
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Ser Gln Arg Ile Ser Thr Leu His His Gln Thr Met Pro Ser Asp Leu
                       25
Thr Gln Asp Glu Phe Thr Gln Leu Ser Gln Ser Ile Ala Glu Phe His
                          40
Thr Tyr Arg Asp Val Asn Val Ile Ser Gly Leu Pro Ala Asn Thr Ser
Arg Glu Arg Leu Asp Leu Leu Asp Asp Asp Arg Arg Val Thr Gly Phe
Ser Ile Thr Gly Gly Glu His Arg Leu Arg Asn Tyr Lys Ser Val Thr
Thr Val His Arg Phe Glu Lys Glu Glu Glu Glu Glu Arg Ile Trp Thr
                              105
Val Val Leu Glu Ser Tyr Val Val Asp Val Pro Glu Gly Asn Ser Glu
               120
Glu Asp Thr Arg Leu Phe Ala Asp Thr Val Ile Arg Leu Asn Leu Gln
               135
Lys Leu Ala Ser Ile Thr Glu Ala Met Asn Arg Asn Asn Asn Asn
                  150
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Asn Ser Ser Gln Val Arg
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<211> LENGTH: 50
<212> TYPE: PRT
<213 > ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: synthetic PYR/PYL receptor polypeptide
     consensus sequence
<221> NAME/KEY: VARIANT
<222> LOCATION: (1) ... (50)
<223> OTHER INFORMATION: Xaa = any amino acid
<400> SEQUENCE: 91
Gly Xaa Xaa Arg Xaa Val Xaa Xaa Xaa Ser Gly Xaa Pro Ala Xaa Xaa
                                 1.0
Ser Xaa Glu Xaa Leu Xaa Xaa Xaa Asp Xaa Xaa Xaa Xaa Xaa Xaa
Xaa Xaa Xaa Xaa Gly Gly Xaa His Arg Leu Xaa Asn Tyr Lys Ser Xaa
                          40
Xaa Xaa
```

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50
<210> SEQ ID NO 92
<211> LENGTH: 41
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: synthetic PYR/PYL receptor polypeptide
     consensus sequence
<221> NAME/KEY: VARIANT
<222> LOCATION: (1) ... (41)
<223> OTHER INFORMATION: Xaa = any amino acid
<400> SEQUENCE: 92
Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Glu Ser Xaa Xaa Val Asp Xaa
Pro Xaa Gly Asn Xaa Xaa Xaa Thr Xaa Xaa Phe Xaa Xaa Xaa
Xaa Xaa Xaa Asn Leu Xaa Xaa Leu Xaa
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<210> SEQ ID NO 93
<211> LENGTH: 36
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: synthetic PYR/PYL receptor polypeptide
    consensus sequence
<221> NAME/KEY: VARIANT
<222> LOCATION: (1) ... (36)
<223> OTHER INFORMATION: Xaa = any amino acid
<400> SEQUENCE: 93
Cys Xaa Ser Xaa Xaa Xaa Xaa Xaa Xaa Ala Pro Xaa Xaa Xaa
                                   10
Trp Xaa Xaa Xaa Xaa Aaa Phe Xaa Xaa Pro Xaa Xaa Xaa Xaa Aaa Phe
                               25
Xaa Xaa Xaa Cys
       35
<210> SEQ ID NO 94
<211> LENGTH: 25
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: synthetic PYR/PYL receptor polypeptide
     consensus sequence
<221> NAME/KEY: VARIANT
<222> LOCATION: (1)...(25)
<223 > OTHER INFORMATION: Xaa = any amino acid
<400> SEQUENCE: 94
Gly Xaa Xaa Arg Xaa Val Xaa Xaa Xaa Ser Xaa Xaa Pro Ala Xaa Xaa
                                  10
Ser Xaa Glu Xaa Leu Xaa Xaa Xaa Asp
           20
<210> SEQ ID NO 95
<211> LENGTH: 11
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: synthetic PYR/PYL receptor polypeptide
     consensus sequence
<221> NAME/KEY: VARIANT
<222> LOCATION: (1) ... (11)
<223 > OTHER INFORMATION: Xaa = any amino acid
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<400> SEQUENCE: 95
Gly Gly Xaa His Arg Leu Xaa Asn Tyr Xaa Ser
<210> SEQ ID NO 96
<211> LENGTH: 36
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: synthetic PYR/PYL receptor polypeptide PYR1 to
     PYL12 consensus sequence
<221> NAME/KEY: VARIANT
<222> LOCATION: (1) ... (36)
<223 > OTHER INFORMATION: Xaa = any amino acid
<400> SEQUENCE: 96
Cys Xaa Ser Xaa Xaa Xaa Xaa Xaa Xaa Ala Pro Xaa Xaa Xaa
Trp Xaa Xaa Xaa Xaa Xaa Phe Xaa Xaa Pro Xaa Xaa Xaa Lys Xaa Phe
                                25
Xaa Xaa Xaa Cys
       35
<210> SEQ ID NO 97
<211> LENGTH: 25
<212> TYPE: PRT
<213 > ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: synthetic PYR/PYL receptor polypeptide PYR1 to
     PYL12 consensus sequence
<221> NAME/KEY: VARIANT
<222> LOCATION: (1)...(25)
<223> OTHER INFORMATION: Xaa = any amino acid
<400> SEOUENCE: 97
Gly Xaa Xaa Arg Xaa Val Xaa Xaa Xaa Ser Xaa Leu Pro Ala Xaa Xaa
                                    10
Ser Xaa Glu Xaa Leu Xaa Xaa Asp
           20
<210> SEQ ID NO 98
<211> LENGTH: 11
<212> TYPE: PRT
<213 > ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: synthetic PYR/PYL receptor polypeptide PYR1 to
     PYL12 consensus sequence
<221> NAME/KEY: VARIANT
<222> LOCATION: (1) ... (11)
<223> OTHER INFORMATION: Xaa = any amino acid
<400> SEQUENCE: 98
Gly Gly Xaa His Arg Leu Xaa Asn Tyr Xaa Ser
<210> SEQ ID NO 99
<211> LENGTH: 31
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: synthetic PYR/PYL receptor polypeptide PYR1 to
    PYL12 consensus sequence
<221> NAME/KEY: VARIANT
<222> LOCATION: (1) ... (31)
<223> OTHER INFORMATION: Xaa = any amino acid
<400> SEQUENCE: 99
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Glu Ser Xaa Xaa Val Asp Xaa Pro Xaa Gly Asn Xaa Xaa Xaa Thr
                                 10
Xaa Xaa Phe Xaa Xaa Xaa Xaa Xaa Xaa Asn Leu Xaa Xaa Leu
           20
                              25
<210> SEQ ID NO 100
<211> LENGTH: 45
<212> TYPE: PRT
<213 > ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: synthetic PYR/PYL receptor polypeptide PYR1 to
    PYL6 consensus sequence
<221> NAME/KEY: VARIANT
<222> LOCATION: (1)...(45)
<223> OTHER INFORMATION: Xaa = any amino acid
<400> SEQUENCE: 100
Xaa Xaa Xaa Ala Pro Xaa Xaa Xaa Trp Xaa Xaa Xaa Xaa Aaa Phe
Xaa Xaa Pro Xaa Xaa Tyr Lys Xaa Phe Xaa Xaa Xaa Cys
<210> SEQ ID NO 101
<211> LENGTH: 48
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: synthetic PYR/PYL receptor polypeptide PYR1 to
   PYL6 consensus sequence
<221> NAME/KEY: VARIANT
<222> LOCATION: (1) ... (48)
<223> OTHER INFORMATION: Xaa = any amino acid
<400> SEQUENCE: 101
Val Gly Arg Xaa Val Xaa Ser Gly Leu Pro Ala Xaa Xaa Ser
1 5
                                10
Xaa Glu Xaa Leu Xaa Xaa Xaa Asp Xaa Xaa Xaa Xaa Xaa Xaa Phe
                              25
Xaa Xaa Xaa Gly Gly Xaa His Arg Leu Xaa Asn Tyr Xaa Ser Val Thr
<210> SEQ ID NO 102
<211> LENGTH: 33
<212> TYPE: PRT
<213 > ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: synthetic PYR/PYL receptor polypeptide PYR1 to
    PYL6 consensus sequence
<221> NAME/KEY: VARIANT
<222> LOCATION: (1) ... (33)
<223 > OTHER INFORMATION: Xaa = any amino acid
<400> SEQUENCE: 102
Val Xaa Glu Ser Tyr Xaa Val Asp Xaa Pro Xaa Gly Asn Xaa Xaa Xaa
Xaa Thr Xaa Xaa Phe Xaa Asp Xaa Xaa Xaa Xaa Asn Leu Gln Xaa
          20
                            25
Leu
<210> SEQ ID NO 103
<211> LENGTH: 50
<212> TYPE: PRT
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<213 > ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: synthetic PYR/PYL receptor polypeptide PYL7 to
     PYL10 consensus sequence
<221> NAME/KEY: VARIANT
<222> LOCATION: (1) ... (50)
<223> OTHER INFORMATION: Xaa = any amino acid
<400> SEQUENCE: 103
His Xaa His Xaa Xaa Xaa Xaa Gln Cys Xaa Ser Xaa Leu Val Lys
Xaa Ile Xaa Ala Pro Xaa His Xaa Val Trp Ser Xaa Val Arg Arg Phe
Asp Xaa Pro Gln Lys Tyr Lys Pro Phe Xaa Ser Arg Cys Xaa Val Xaa 35 40 45
Gly Xaa
<210> SEQ ID NO 104
<211> LENGTH: 65
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: synthetic PYR/PYL receptor polypeptide PYL7 to
    PYL10 consensus sequence
<221> NAME/KEY: VARIANT
<222> LOCATION: (1) ... (65)
<223> OTHER INFORMATION: Xaa = any amino acid
<400> SEQUENCE: 104
Glu Xaa Gly Xaa Xaa Arg Glu Val Xaa Xaa Lys Ser Gly Leu Pro Ala
Thr Xaa Ser Thr Glu Xaa Leu Glu Xaa Leu Asp Asp Xaa Glu His Ile
                                25
Leu Xaa Ile Xaa Ile Xaa Gly Gly Asp His Arg Leu Lys Asn Tyr Ser
Ser Xaa Xaa Xaa His Xaa Glu Xaa Ile Xaa Gly Xaa Xaa Gly Thr
                       55
Xaa
<210> SEQ ID NO 105
<211> LENGTH: 40
<212> TYPE: PRT
<213 > ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: synthetic PYR/PYL receptor polypeptide PYL7 to
     PYL10 consensus sequence
<221> NAME/KEY: VARIANT
<222> LOCATION: (1) ... (40)
<223 > OTHER INFORMATION: Xaa = any amino acid
<400> SEQUENCE: 105
Xaa Xaa Glu Ser Phe Val Val Asp Val Pro Xaa Gly Asn Thr Lys Xaa
      5
                                   10
Xaa Thr Cys Xaa Phe Val Glu Xaa Leu Ile Xaa Cys Asn Leu Xaa Ser
                       25
Leu Ala Xaa Xaa Glu Arg Leu
      35
<210> SEQ ID NO 106
<211> LENGTH: 44
<212> TYPE: PRT
<213 > ORGANISM: Artificial Sequence
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<220> FEATURE:
<223> OTHER INFORMATION: synthetic PYR/PYL receptor polypeptide PYL11 to
     PYL13 consensus sequence
<221> NAME/KEY: VARIANT
<222> LOCATION: (1) ... (44)
<223> OTHER INFORMATION: Xaa = any amino acid
<400> SEQUENCE: 106
Cys Xaa Ser Xaa Xaa Val Xaa Thr Ile Xaa Ala Pro Leu Xaa Leu Val
Trp Ser Ile Leu Arg Xaa Phe Asp Xaa Pro Xaa Xaa Xaa Xaa Xaa Phe
Val Lys Xaa Cys Xaa Xaa Xaa Ser Gly Xaa Gly Gly
<210> SEQ ID NO 107
<211> LENGTH: 49
<212> TYPE: PRT
<213 > ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: synthetic PYR/PYL receptor polypeptide PYL11 to
     PYL13 consensus sequence
<221> NAME/KEY: VARIANT
<222> LOCATION: (1) ... (49)
<223> OTHER INFORMATION: Xaa = any amino acid
<400> SEQUENCE: 107
Gly Ser Val Arg Xaa Val Thr Xaa Val Ser Xaa Xaa Pro Ala Xaa Phe
Ser Xaa Glu Arg Leu Xaa Glu Leu Asp Asp Glu Ser His Val Met Xaa
Xaa Ser Ile Ile Gly Gly Xaa His Arg Leu Val Asn Tyr Xaa Ser Lys
                           40
Thr
<210> SEQ ID NO 108
<211> LENGTH: 188
<212> TYPE: PRT
<213 > ORGANISM: Zea mays
<220> FEATURE:
<223> OTHER INFORMATION: corn (maize) PYR/PYL receptor polypeptide
<400> SEQUENCE: 108
Met Glu Pro His Met Glu Ser Ala Leu Arg Gln Gly Leu Ser Glu Ala
Glu Gln Arg Glu Leu Glu Gly Val Val Arg Ala His His Thr Phe Pro
Gly Arg Ala Pro Gly Thr Cys Thr Ser Leu Val Thr Gln Arg Val Asp
Ala Pro Leu Ala Ala Val Trp Pro Ile Val Arg Gly Phe Gly Ser Pro
Gln Arg Tyr Lys His Phe Ile Lys Ser Cys Asp Leu Lys Ala Gly Asp
Gly Ala Thr Val Gly Ser Val Arg Glu Val Thr Val Val Ser Gly Leu
Pro Ala Ser Thr Ser Thr Glu Arg Leu Glu Ile Leu Asp Asp His Arg
                               105
His Ile Leu Ser Phe Arg Val Val Gly Gly Asp His Arg Leu Arg Asn
                          120
                                              125
Tyr Arg Ser Val Thr Ser Val Thr Glu Phe Gln Pro Gly Pro Tyr Cys
               135
                                        140
```

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Val Val Leu Glu Ser Tyr Val Val Asp Val Pro Asp Gly Asn Thr Glu 155 Glu Asp Thr Arg Met Phe Thr Asp Thr Val Val Lys Leu Asn Leu Gln 165 170 Lys Leu Ala Ala Ile Ala Thr Ser Ser Ser Ala Asn <210> SEQ ID NO 109 <211> LENGTH: 205 <212> TYPE: PRT <213> ORGANISM: Zea mays <220> FEATURE: <223> OTHER INFORMATION: corn (maize) PYR/PYL receptor polypeptide <400> SEQUENCE: 109 Met Asp Gln Gln Gly Ala Gly Gly Asp Val Glu Val Pro Ala Gly Leu Gly Leu Thr Ala Ala Glu Tyr Glu Gln Leu Arg Pro Thr Val Asp Ala His His Arg Tyr Ala Val Gly Glu Gly Gln Cys Ser Ser Leu Leu Ala Gln Arg Ile His Ala Pro Pro Ala Ala Val Trp Ala Ile Val Arg Arg Phe Asp Cys Pro Gln Val Tyr Lys His Phe Ile Arg Ser Cys Ala Val Arg Pro Asp Pro Asp Ala Gly Asp Ala Leu Arg Pro Gly Arg Leu Arg Glu Val Cys Val Ile Ser Gly Leu Pro Ala Ser Thr Ser Thr Glu Arg 100 105 Leu Asp His Leu Asp Asp Ala Ala Arg Val Phe Gly Phe Ser Ile Thr 120 Gly Glu His Arg Leu Arg Asn Tyr Arg Ser Val Thr Thr Val Ser Glu Leu Ala Gly Pro Gly Ile Cys Thr Val Val Leu Glu Ser Tyr Ala 150 155 Val Asp Val Pro Asp Gly Asn Thr Glu Asp Asp Thr Arg Leu Phe Ala Asp Thr Val Ile Arg Leu Asn Leu Gln Lys Leu Lys Ser Val Ala Glu 185 Ala Ser Thr Ser Ser Ser Ala Pro Pro Pro Pro Ser Glu <210> SEQ ID NO 110 <211> LENGTH: 220 <212> TYPE: PRT <213 > ORGANISM: Zea mays <220> FEATURE: <223> OTHER INFORMATION: corn (maize) PYR/PYL receptor polypeptide <400> SEQUENCE: 110 Met Pro Cys Ile Gln Ala Ser Ser Pro Gly Gly Met Pro His Gln His 1.0 Gly Arg Gly Arg Val Leu Gly Gly Gly Val Gly Cys Ala Ala Glu Val Ala Ala Ala Val Ala Ala Ser Ala Gly Gly Met Arg Cys Gly Ala His

Asp Gly Glu Val Pro Ala Glu Ala Ala Arg His His Glu His Ala Ala

_	50					55					60				
	50					55					60				
Ala 65	Gly	Pro	Gly	Arg	Сув 70	Cys	Ser	Ala	Val	Val 75	Gln	His	Val	Ala	Ala 80
Pro	Ala	Ala	Ala	Val 85	Trp	Ser	Val	Val	Arg 90	Arg	Phe	Asp	Gln	Pro 95	Gln
Val	Tyr	Lys	Arg 100	Phe	Val	Arg	Ser	Cys 105	Ala	Leu	Leu	Ala	Gly 110	Asp	Gly
Gly	Val	Gly 115	Thr	Leu	Arg	Glu	Val 120	Arg	Val	Val	Ser	Gly 125	Leu	Pro	Ala
Ala	Ser 130	Ser	Arg	Glu	Arg	Leu 135	Glu	Val	Leu	Asp	Asp 140	Glu	Ser	His	Val
Leu 145	Ser	Phe	Arg	Val	Val 150	Gly	Gly	Glu	His	Arg 155	Leu	Arg	Asn	Tyr	Leu 160
Ser	Val	Thr	Thr	Val 165	His	Pro	Ser	Pro	Ala 170	Ala	Pro	Asp	Ala	Ala 175	Thr
Val	Val	Val	Glu 180	Ser	Tyr	Val	Val	Asp 185	Val	Pro	Pro	Gly	Asn 190	Thr	Pro
Glu	Asp	Thr 195	Arg	Val	Phe	Val	Asp 200	Thr	Ile	Val	Lys	Сув 205	Asn	Leu	Gln
Ser	Leu 210	Ala	Thr	Thr	Ala	Glu 215	Lys	Leu	Ala	Ala	Val 220				
<213 <213 <220 <223		PE: RGANI ATUR HER	PRT ISM: RE: INFO	Glyo DRMA:			/bear	ı PYI	R/PYI	i red	cepto	or po	olype	eptic	le
<400	)> SE	TOOFI	NCE:	111											
Met 1	Glu	Lys	Ala	Glu 5	Ser	Ser	Ala	Ser	Thr 10	Ser	Glu	Pro	Asp	Ser 15	Asp
Glu	Asn	His	His 20	Arg	His	Pro	Thr	Asn 25	His	His	Ile	Asn	Pro 30	Pro	Ser
Gly	Leu	Thr 35	Pro	Leu	Glu	Phe	Ala 40	Ser	Leu	Ile	Pro	Ser 45	Val	Ala	Glu
His	His 50	Ser	Tyr	Leu	Val	Gly 55	Ser	Gly	Gln	Сла	Ser 60	Ser	Leu	Leu	Ala
Gln 65	Arg	Val	Gln	Ala	Pro 70	Pro	Asp	Ala	Val	Trp 75	Ser	Val	Val	Arg	Arg 80
Phe	Asp	Lys	Pro	Gln 85	Thr	Tyr	Lys	His	Phe 90	Ile	Lys	Ser	Cys	Ala 95	Val
Lys	Glu	Pro	Phe 100	His	Met	Ala	Val	Gly 105	Val	Thr	Arg	Asp	Val 110	Asn	Val
Ile	Ser	Gly 115	Leu	Pro	Ala	Ala	Thr 120	Ser	Thr	Glu	Arg	Leu 125	Asp	Leu	Leu
Asp	Asp 130	Ile	Arg	CAa	Val	Thr 135	Gly	Phe	Ser	Ile	Ile 140	Gly	Gly	Glu	His
Arg 145	Leu	Arg	Asn	Tyr	Arg 150	Ser	Val	Thr	Thr	Val 155	His	Ser	Phe	Glu	Asp 160
Asp	Ala	Asp	Asp	Gly 165	Lys	Ile	Tyr	Thr	Val 170	Val	Leu	Glu	Ser	Tyr 175	Val
Val	Asp	Val	Pro 180	Asp	Gly	Asn	Thr	Glu 185	Glu	Asp	Thr	Arg	Leu 190	Phe	Ala
Asp	Thr	Val	Val	ГЛа	Leu	Asn	Leu	Gln	Lys	Leu	Ala	Ser	Val	Thr	Glu

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195
                           200
Gly Thr Asn Arg Asp Gly Asp Gly Lys Ser His Ser Arg
                       215
<210> SEQ ID NO 112
<211> LENGTH: 214
<212> TYPE: PRT
<213> ORGANISM: Glycine max
<220> FEATURE:
<223> OTHER INFORMATION: soybean PYR/PYL receptor polypeptide
<400> SEQUENCE: 112
Met Glu Lys Thr His Ser Ser Ser Ala Glu Glu Gln Asp Pro Thr Arg
Arg His Leu Asp Pro Pro Pro Gly Leu Thr Ala Glu Glu Phe Glu Asp
Leu Lys Pro Ser Val Leu Glu His His Thr Tyr Ser Val Thr Pro Thr
Arg Gln Ser Ser Ser Leu Leu Ala Gln Arg Ile His Ala Pro Pro His
Ala Val Trp Ser Val Val Arg Cys Phe Asp Asn Pro Gln Ala Tyr Lys 65 70 75 80
His Phe Ile Lys Ser Cys His Val Lys Glu Gly Phe Gln Leu Ala Val
              85
                                  90
Gly Ser Thr Arg Asp Val His Val Ile Ser Gly Leu Pro Ala Ala Thr
                              105
Ser Thr Glu Arg Leu Asp Leu Leu Asp Asp Asp Arg His Val Ile Gly
Phe Thr Ile Val Gly Gly Asp His Arg Leu Arg Asn Tyr Arg Ser Val
Thr Ser Val His Gly Phe Glu Cys Asp Gly Lys Ile Trp Thr Val Val
                 150
Leu Glu Ser Tyr Val Val Asp Val Pro Glu Gly Asn Thr Glu Glu Asp
Thr Arg Leu Phe Ala Asp Thr Val Val Lys Leu Asn Leu Gln Lys Leu
                              185
Ala Ser Val Ser Glu Gly Met Cys Gly Asp Gly Asp Gly Asp
Gly Lys Gly Asn Lys Ser
<210> SEQ ID NO 113
<211> LENGTH: 216
<212> TYPE: PRT
<213> ORGANISM: Glycine max
<220> FEATURE:
<223> OTHER INFORMATION: soybean PYR/PYL receptor polypeptide
<400> SEQUENCE: 113
Met Leu Gln Asn Ser Ser Met Ser Ser Leu Leu His Arg Ile Asn
Gly Gly Gly Ala Thr Thr Ala Thr Asn Cys His Asp Thr Val Phe
                              25
Met Thr Val Pro Asp Gly Val Ala Arg Tyr His Thr His Ala Val Ala
                40
Pro Asn Gln Cys Cys Ser Ser Val Ala Gln Glu Ile Gly Ala Ser Val
                       55
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Ala 65	Thr	Val	Trp	Ser	Val 70	Leu	Arg	Arg	Phe	Asp 75	Asn	Pro	Gln	Ala	Tyr 80
Lys	His	Phe	Val	Lys 85	Ser	CAa	His	Val	Ile 90	Gly	Gly	Asp	Gly	Asp 95	Val
Gly	Thr	Leu	Arg 100	Glu	Val	His	Val	Ile 105	Ser	Gly	Leu	Pro	Ala 110	Ala	Arg
Ser	Thr	Glu 115	Arg	Leu	Glu	Ile	Leu 120	Asp	Asp	Glu	Arg	His 125	Val	Ile	Ser
Phe	Ser 130	Val	Val	Gly	Gly	Asp 135	His	Arg	Leu	Ala	Asn 140	Tyr	Arg	Ser	Val
Thr 145	Thr	Leu	His	Pro	Thr 150	Ala	Ser	Ser	Ala	Ser 155	Gly	Gly	Cys	Ser	Gly 160
Thr	Val	Val	Val	Glu 165	Ser	Tyr	Val	Val	Asp 170	Val	Pro	Pro	Gly	Asn 175	Thr
Arg	Glu	Asp	Thr 180	Arg	Val	Phe	Val	Asp 185	Thr	Ile	Val	Lys	Cys 190	Asn	Leu
Gln	Ser	Leu 195	Ala	Gln	Thr	Ala	Glu 200	Asn	Leu	Thr	Leu	Arg 205	Lys	Asn	Asn
Asn	Asn 210	Asp	Tyr	Lys	CAa	Cys 215	Ser								
<213 <213 <213 <220	0 > SI 1 > LI 2 > TY 3 > OF 0 > FI 3 > O	ENGTI (PE : RGAN: EATUI	H: 20 PRT ISM: RE:	08 Gly			ybear	ı PYI	R/PYI	i re	cepto	or po	olype	∍ptio	de
< 40	0> SI	EQUEI	ICE :	114											
Met 1	Thr	Ser	Leu	Gln 5	Phe	His	Arg	Phe	Asn 10	Pro	Ala	Thr	Asp	Thr 15	Ser
Thr	Ala	Ile	Ala 20	Asn	Gly	Val	Asn	Сув 25	Pro	Lys	Pro	Pro	Ser 30	Thr	Leu
Arg	Leu	Leu 35	Ala	Lys	Val	Ser	Leu 40	Ser	Val	Pro	Glu	Thr 45	Val	Ala	Arg
His	His 50	Ala	His	Pro	Val	Gly 55	Pro	Asn	Gln	СЛа	Cys	Ser	Val	Val	Ile
Gln 65	Ala	Ile	Asp	Ala	Pro 70	Val	Ser	Ala	Val	Trp 75	Pro	Val	Val	Arg	Arg 80
Phe	Asp	Asn	Pro	Gln 85	Ala	Tyr	Lys	His	Phe 90	Val	Lys	Ser	Сув	His 95	Val
Val	Ala	Ala	Ala 100	Gly	Gly	Gly	Glu	Asp 105	Gly	Ile	Arg	Val	Gly 110	Ala	Leu
Arg	Glu	Val 115	Arg	Val	Val	Ser	Gly 120	Leu	Pro	Ala	Val	Ser 125	Ser	Thr	Glu
Arg	Leu 130	Glu	Ile	Leu	Asp	Asp 135	Glu	Arg	His	Val	Met 140	Ser	Phe	Ser	Val
Val 145	Gly	Gly	Asp	His	Arg 150	Leu	Arg	Asn	Tyr	Arg 155	Ser	Val	Thr	Thr	Leu 160
His	Gly	Asp	Gly	Asn 165	Gly	Gly	Thr	Val	Val 170	Ile	Glu	Ser	Tyr	Val 175	Val
Asp	Val	Pro	Pro 180	Gly	Asn	Thr	Lys	Glu 185	Glu	Thr	Cys	Val	Phe	Val	Asp
Thr	Ile	Val 195	Arg	CÀa	Asn	Leu	Gln 200	Ser	Leu	Ala	Gln	Ile 205	Ala	Glu	Thr

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<210> SEQ ID NO 115
<211> LENGTH: 176
<212> TYPE: PRT
<213> ORGANISM: Glycine max
<220> FEATURE:
<223> OTHER INFORMATION: soybean PYR/PYL receptor polypeptide
<400> SEQUENCE: 115
Ala Tyr Pro Val Leu Gly Leu Thr Pro Glu Glu Phe Ser Glu Leu Glu
Ser Ile Ile Asn Thr His His Lys Phe Glu Pro Ser Pro Glu Ile Cys
Ser Ser Ile Ile Ala Gln Arg Ile Asp Ala Pro Ala His Thr Val Trp
Pro Leu Val Arg Ser Phe Glu Asn Pro Gln Lys Tyr Lys His Phe Val
Lys Ser Cys Asn Met Arg Ser Gly Asp Gly Gly Val Gly Ser Ile Arg
Glu Val Thr Val Val Ser Gly Leu Pro Ala Ser Thr Ser Thr Glu Arg
                                 90
Leu Glu Ile Leu Asp Asp Asp Lys His Leu Leu Ser Phe Arg Val Val
                               105
Gly Gly Glu His Arg Leu His Asn Tyr Arg Ser Val Thr Ser Val Asn
                           120
Glu Phe Lys Asn Pro Asp Asn Gly Lys Val Tyr Thr Ile Val Leu Glu
            135
Ser Tyr Val Val Asp Ile Pro Glu Gly Asn Thr Gly Val Asp Thr Lys
                  150
                                      155
Met Phe Val Asp Thr Val Val Lys Leu Asn Leu Gln Lys Leu Gly Glu
<210> SEQ ID NO 116
<211> LENGTH: 172
<212> TYPE: PRT
<213> ORGANISM: Glycine max
<220> FEATURE:
<223> OTHER INFORMATION: soybean PYR/PYL receptor polypeptide
<400> SEQUENCE: 116
Glu Phe Thr Glu Leu Glu Ser Thr Ile Asn Thr His His Lys Phe Glu
Ala Ser Pro Glu Ile Cys Ser Ser Ile Ile Ala Gln Arg Ile Asp Ala
Pro Ala His Thr Val Trp Pro Leu Val Arg Ser Phe Glu Asn Pro Gln
Lys Tyr Lys His Phe Val Lys Ser Cys Asn Met Arg Ser Gly Asp Gly
Gly Val Gly Ser Ile Arg Glu Val Thr Val Val Ser Gly Leu Pro Ala
Ser Thr Ser Thr Glu Arg Leu Glu Ile Leu Asp Asp Asp Asn His Leu
Leu Ser Phe Arg Val Val Gly Glu His Arg Leu His Asn Tyr Arg
                               105
Ser Val Thr Ser Val Asn Glu Phe Lys Arg Pro Asp Asn Gly Lys Val
                          120
                                               125
Tyr Thr Ile Val Leu Glu Ser Tyr Val Val Asp Ile Pro Glu Gly Asn
               135
                                         140
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Thr Gly Val Asp Thr Lys Met Phe Val Asp Thr Val Val Lys Leu Asn 150 Leu Gln Lys Leu Gly Glu Val Ala Met Ala Thr Asn <210> SEQ ID NO 117 <211> LENGTH: 191 <212> TYPE: PRT <213 > ORGANISM: Glycine max <220> FEATURE: <223> OTHER INFORMATION: soybean PYR/PYL receptor polypeptide <400> SEQUENCE: 117 Met Thr Glu Leu Ser Ser Arg Glu Val Glu Tyr Ile Arg Arg His His Ser Lys Ala Ala Glu Asp Asn Gln Cys Ala Ser Ala Leu Val Lys His Ile Arg Ala Pro Leu Pro Leu Val Trp Ser Leu Val Arg Arg Phe Asp Glu Pro Gln Lys Tyr Lys Pro Phe Val Ser Arg Cys Val Val Arg Gly 55 Asn Leu Glu Ile Gly Ser Leu Arg Glu Val Asp Val Lys Ser Gly Leu Pro Ala Thr Thr Ser Thr Glu Arg Leu Glu Ile Leu Asp Asp Asn His His Ile Leu Ser Val Arg Ile Ile Gly Gly Asp His Arg Leu Arg Asn 105 Tyr Ser Ser Ile Met Ser Leu His Pro Glu Ile Val Asp Gly Arg Pro 120 Gly Thr Leu Val Ile Glu Ser Phe Val Val Asp Ile Pro Glu Gly Asn 135 Thr Lys Asp Glu Thr Cys Tyr Phe Val Glu Ala Leu Ile Lys Cys Asn Leu Lys Ser Leu Ala Asp Val Ser Glu Gly Leu Thr Leu Gln Asp His 170 Thr Glu Pro Ile Asp Arg Lys Tyr Glu Leu Leu Ile Thr Arg Gly <210> SEQ ID NO 118 <211> LENGTH: 185 <212> TYPE: PRT <213> ORGANISM: Glycine max <223> OTHER INFORMATION: soybean PYR/PYL receptor polypeptide <400> SEQUENCE: 118 Met Asn Gly Gly Glu Ser Tyr Gly Ala Ile Glu Thr Gln Tyr Ile Arg Arg His His Lys His Glu Pro Arg Glu Asn Gln Cys Thr Ser Ala Leu 25 Val Lys His Ile Arg Ala Pro Val His Leu Val Trp Ser Leu Val Arg 40 Arg Phe Asp Gln Pro Gln Lys Tyr Lys Pro Phe Val Ser Arg Cys Ile Met Gln Gly Asp Leu Gly Ile Gly Ser Val Arg Glu Val Asn Val Lys Ser Gly Leu Pro Ala Thr Thr Ser Thr Glu Arg Leu Glu Gln Leu Asp

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90 Asp Glu Glu His Ile Leu Gly Ile Arg Ile Val Gly Gly Asp His Arg 105 Leu Arg Asn Tyr Ser Ser Ile Ile Thr Val His Pro Glu Val Ile Asp 120 Gly Arg Pro Gly Thr Met Val Ile Glu Ser Phe Val Val Asp Val Pro 135 Asp Gly Asn Thr Arg Asp Glu Thr Cys Tyr Phe Val Glu Ala Leu Ile Arg Cys Asn Leu Ser Ser Leu Ala Asp Val Ser Glu Arg Met Ala Val Gln Gly Arg Thr Asn Pro Ile Asn His <210> SEQ ID NO 119 <211> LENGTH: 178 <212> TYPE: PRT <213> ORGANISM: Glycine max <220> FEATURE: <223> OTHER INFORMATION: soybean PYR/PYL receptor polypeptide <400> SEOUENCE: 119 Met Ser Pro Asn Asn Pro Ser Thr Ile Val Ser Asp Ala Val Ala Arg His His Thr His Val Val Ser Pro His Gln Cys Cys Ser Ala Val Val 20 25 Gln Glu Ile Ala Ala Pro Val Ser Thr Val Trp Ser Val Val Arg Arg 40 Phe Asp Asn Pro Gln Ala Tyr Lys His Phe Val Lys Ser Cys His Val Ile Leu Gly Asp Gly Asp Val Gly Thr Leu Arg Glu Val Arg Val Ile Ser Gly Leu Pro Ala Ala Val Ser Thr Glu Arg Leu Asp Val Leu Asp Asp Glu Arg His Val Ile Gly Phe Ser Met Val Gly Gly Asp His Arg Leu Ser Asn Tyr Arg Ser Val Thr Ile Leu His Pro Arg Ser Ala Thr 120 Asp Thr Val Val Val Glu Ser Tyr Val Val Asp Val Pro Ala Gly Asn Thr Thr Glu Asp Thr Arg Val Phe Val Asp Thr Ile Leu Arg Cys Asn Leu Gln Ser Leu Ala Lys Phe Ala Glu Asn Leu Thr Asn Lys Leu His Gln Arg <210> SEQ ID NO 120 <211> LENGTH: 246 <212> TYPE: PRT <213> ORGANISM: Glycine max <220> FEATURE: <223> OTHER INFORMATION: soybean PYR/PYL receptor polypeptide <400> SEQUENCE: 120 Met Ser Arg Ser His Asn Lys Arg Lys Pro Phe Ser Phe Ile Phe Lys 1 5

Ile Thr Leu Leu Glu Leu Leu Ser Ser Leu Leu Ser Ser Ser Leu Arg

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Thr	His 50	Pro	Thr	Arg	Asn	His 55	Leu	Asp	Pro	Pro	Pro 60	Gly	Leu	Thr	Pro
Glu 65	Glu	Phe	Glu	Asp	Leu 70	Lys	Pro	Ser	Val	Leu 75	Glu	His	His	Thr	Tyr 80
Ser	Val	Thr	Pro	Thr 85	Arg	Gln	Cys	Ser	Ser 90	Leu	Leu	Ala	Gln	Arg 95	Ile
His	Ala	Pro	Pro 100	His	Thr	Val	Trp	Thr 105	Val	Val	Arg	Cya	Phe 110	Asp	Asn
Pro	Gln	Ala 115	Tyr	ГÀа	His	Phe	Ile 120	Lys	Ser	Cys	His	Val 125	Lys	Glu	Gly
Phe	Gln 130	Leu	Ala	Val	Gly	Ser 135	Thr	Arg	Asp	Val	His 140	Val	Ile	Ser	Gly
Leu 145	Pro	Ala	Ala	Thr	Ser 150	Thr	Glu	Arg	Leu	Asp 155	Leu	Leu	Asp	Asp	Asp 160
Arg	His	Val	Ile	Gly 165	Phe	Thr	Ile	Val	Gly 170	Gly	Asp	His	Arg	Leu 175	Arg
Asn	Tyr	Arg	Ser 180	Val	Thr	Ser	Val	His 185	Gly	Phe	Glu	Arg	Asp 190	Gly	Lys
Ile	Trp	Thr 195	Val	Val	Leu	Glu	Ser 200	Tyr	Val	Val	Asp	Val 205	Pro	Glu	Gly
Asn	Thr 210	Glu	Glu	Asp	Thr	Arg 215	Leu	Phe	Ala	Asp	Thr 220	Val	Val	ГЛа	Leu
Asn 225	Leu	Gln	Lys	Leu	Ala 230	Ser	Val	Thr	Glu	Gly 235	Met	CAa	Gly	Asp	Ser 240
Asp	Gly	Lys	Gly	Asn 245	Asn										
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Arg 145	Leu	Arg	Asn	Tyr	Arg 150	Ser	Val	Thr	Thr	Val 155	His	Ser	Phe	Asp	Asp 160
Asp	Asn	Ala	Ser	Ala 165	Asp	Gly	Lys	Ile	Tyr 170	Thr	Val	Val	Leu	Glu 175	Ser
Tyr	Val	Val	Asp 180	Val	Pro	Asp	Gly	Asn 185	Thr	Glu	Glu	Asp	Thr 190	Arg	Leu
Phe	Ala	Asp 195	Thr	Val	Val	Lys	Leu 200	Asn	Leu	Gln	Lys	Leu 205	Ala	Ser	Val
Thr	Glu 210	Gly	Thr	Asn	Gly	Asp 215	Gly	Asp	Gly	Lys	Pro 220	His	Ser	Arg	
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Gln	Ala	Pro 35	Pro	Ser	Ser	Thr	Ala 40	Ala	Arg	Arg	Leu	Val 45	Val	Pro	Ser
Leu	Ser 50	Ser	Gly	Arg	Gly	Ile 55	Ala	Ala	Pro	Asp	Thr 60	Val	Ala	Leu	His
His 65	Ala	His	Val	Val	Asp 70	Pro	Asn	Gln	Cys	Сув 75	Ser	Ile	Val	Thr	Gln 80
His	Ile	Asn	Ala	Pro 85	Val	Ser	Ala	Val	Trp 90	Ala	Val	Val	Arg	Arg 95	Phe
Asp	Asn	Pro	Gln 100	Gly	Tyr	Lys	Asn	Phe 105	Val	Arg	Ser	Сув	His 110	Val	Ile
Thr	Gly	Asp 115	Gly	Ile	Arg	Val	Gly 120	Ala	Val	Arg	Glu	Val 125	Arg	Val	Val
Ser	Gly 130	Leu	Pro	Ala	Glu	Thr 135	Ser	Thr	Glu	Arg	Leu 140	Glu	Ile	Leu	Asp
Asp 145	Glu	Arg	His	Val	Ile 150	Ser	Phe	Ser	Met	Val 155	Gly	Gly	Asp	His	Arg 160
Leu	Arg	Asn	Tyr	Gln 165	Ser	Val	Thr	Thr	Leu 170	His	Ala	Asn	Gly	Asn 175	Gly
Thr	Leu	Val	Ile 180	Glu	Ser	Tyr	Val	Val 185	Asp	Val	Pro	Gln	Gly 190	Asn	Thr
Lys	Glu	Glu 195	Thr	CAa	Val	Phe	Val 200	Asp	Thr	Ile	Val	Arg 205	Cys	Asn	Leu
Gln	Ser 210	Leu	Ala	Gln	Ile	Ala 215	Glu	Asn	Arg	Thr	Asn 220	Asn	Cys	Glu	His
Thr 225	Ala	Gln	His	Cys											
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Ala Gln Met Ala Glu Asn Met Gly Ser
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<220> FEATURE:
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Ile Ala His Gln Asn Tyr Met Ala Ser Glu Thr His His His Val Gln
Gly Leu Thr Pro Glu Glu Leu Thr Lys Leu Glu Pro Ile Ile Lys Lys
Tyr His Leu Phe Glu Gln Ser Pro Asn Thr Cys Phe Ser Ile Ile Thr
           55
Tyr Arg Ile Glu Ala Pro Ala Lys Ala Val Trp Pro Phe Val Arg Ser
Phe Asp Asn Pro Gln Lys Tyr Lys His Phe Ile Lys Gly Cys Asn Met
Arg Gly Asp Gly Gly Val Gly Ser Ile Arg Glu Val Thr Val Val Ser
Gly Leu Pro Ala Ser Thr Ser Thr Glu Arg Leu Glu Ile Leu Asp Asp
                         120
Asp Lys His Val Leu Ser Phe Arg Val Val Gly Gly Glu His Arg Leu
                      135
Lys Asn Tyr Arg Ser Val Thr Ser Val Asn Glu Phe Asn Lys Glu Gly
Lys Val Tyr Thr Ile Val Leu Glu Ser Tyr Ile Val Asp Ile Pro Glu
Gly Asn Thr Glu Glu Asp Thr Lys Met Phe Val Asp Thr Val Val Lys
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Leu Asn Leu Gln Lys Leu Gly Val Val Ala Met Ala Ser Ser Met His
                           200
Gly Gln
<210> SEQ ID NO 126
<211> LENGTH: 193
<212> TYPE: PRT
<213 > ORGANISM: Glycine max
<220> FEATURE:
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Met Asn Arg Ile Gly Asn Gly Gly Gly Gly Gly Gly Leu Ser Asn
Val Glu Met Glu Tyr Ile Arg Arg His His Arg His Glu Pro Gly Glu
Asn Gln Cys Gly Ser Ala Leu Val Lys His Ile Arg Ala Pro Val Pro
Gln Val Trp Ser Leu Val Arg Arg Phe Asp Gln Pro Gln Lys Tyr Lys
                       55
Pro Phe Ile Ser Arg Cys Val Val Arg Gly Asn Leu Glu Ile Gly Ser
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Leu Arg Glu Val Asp Val Lys Ser Gly Leu Pro Ala Thr Thr Ser Thr Glu Arg Leu Glu Leu Leu Asp Asp Asn Glu His Ile Leu Ser Ile Arg 105 Ile Ile Gly Gly Asp His Arg Leu Arg Asn Tyr Ser Ser Ile Met Ser Leu His Pro Glu Ile Ile Asp Gly Arg Pro Gly Thr Leu Val Ile Glu Ser Phe Val Val Asp Val Pro Glu Gly Asn Thr Lys Asp Glu Thr Cys Tyr Phe Val Glu Ala Leu Ile Lys Cys Asn Leu Lys Ser Leu Ala Asp Val Ser Glu Gly Leu Ala Val Gln Asp Cys Thr Glu Pro Ile Asp Arg Ile <210> SEQ ID NO 127 <211> LENGTH: 188 <212> TYPE: PRT <213 > ORGANISM: Glycine max <220> FEATURE: <223> OTHER INFORMATION: soybean PYR/PYL receptor polypeptide <400> SEQUENCE: 127  $\hbox{Met Ala Ser Glu Thr His His His Val Gln Gly Leu Thr Pro Glu Glu } \\$ Leu Thr Gln Leu Glu Pro Ile Ile Lys Lys Tyr His Leu Phe Glu Ala Ser Ser Asn Lys Cys Phe Ser Ile Ile Thr His Arg Ile Glu Ala Pro Ala Ser Ser Val Trp Pro Leu Val Arg Asn Phe Asp Asn Pro Gln Lys Tyr Lys His Phe Ile Lys Gly Cys Asn Met Lys Gly Asp Gly Ser Val Gly Ser Ile Arg Glu Val Thr Val Val Ser Gly Leu Pro Ala Ser Thr Ser Thr Glu Arg Leu Glu Ile Leu Asp Asp Asp Lys His Val Leu Ser Phe Arg Val Val Gly Gly Glu His Arg Leu Gln Asn Tyr Arg Ser Val Thr Ser Val Asn Glu Phe His Lys Glu Gly Lys Val Tyr Thr Ile Val Thr Lys Met Phe Val Asp Thr Val Val Lys Leu Asn Leu Gln Lys Leu 170 Gly Val Val Ala Met Ala Ser Ser Met Asn Gly Arg <210> SEQ ID NO 128 <211> LENGTH: 177 <212> TYPE: PRT <213 > ORGANISM: Glycine max <220> FEATURE: <223> OTHER INFORMATION: soybean PYR/PYL receptor polypeptide

<400> SEQUENCE: 128

Met Leu Pro Asn Asn Pro Ser Thr Ile Val Pro Asp Ala Val Ala Arg

His His Thr His Val Val Ser Pro Gln Gln Cys Cys Ser Ala Val Val Gln Glu Ile Ala Ala Pro Val Ser Thr Val Trp Ser Val Val Arg Arg Phe Asp Asn Pro Gln Ala Tyr Lys His Phe Val Lys Ser Cys His Val Ile Leu Gly Asp Gly Asp Val Gly Thr Leu Arg Glu Val His Val Ile Ser Gly Leu Pro Ala Ala Val Ser Thr Glu Arg Leu Asp Val Leu Asp Asp Glu Arg His Val Ile Gly Phe Ser Met Val Gly Gly Asp His Arg Leu Phe Asn Tyr Arg Ser Val Thr Thr Leu His Pro Arg Ser Ala Ala Gly Thr Val Val Val Glu Ser Tyr Val Val Asp Val Pro Pro Gly Asn 135 Thr Thr Glu Asp Thr Arg Val Phe Val Asp Thr Ile Leu Arg Cys Asn 150 Leu Gln Ser Leu Ala Lys Phe Ala Glu Asn Leu Thr Lys Leu His Gln Arg <210> SEQ ID NO 129 <211> LENGTH: 185 <212> TYPE: PRT <213> ORGANISM: Glycine max <220> FEATURE: <223> OTHER INFORMATION: soybean PYR/PYL receptor polypeptide <400> SEQUENCE: 129 Met Asn Gly Gly Glu Ser Tyr Gly Ala Ile Glu Thr Gln Tyr Ile Arg Arg His His Lys His Glu Pro Arg Glu Asn Gln Cys Thr Ser Ala Leu Val Lys His Ile Arg Ala Pro Val His Leu Val Trp Ser Leu Val Arg Arg Phe Asp Gln Pro Gln Lys Tyr Lys Pro Phe Val Ser Arg Cys Ile Met Gln Gly Asp Leu Gly Ile Gly Ser Val Arg Glu Val Asn Val Lys Ser Gly Leu Pro Ala Thr Thr Ser Thr Glu Arg Leu Glu Gln Leu Asp Asp Glu Glu His Ile Leu Gly Ile Arg Ile Val Gly Gly Asp His Arg 105 Leu Arg Asn Tyr Ser Ser Ile Ile Thr Val His Pro Glu Val Ile Asp Gly Arg Pro Gly Thr Met Val Ile Glu Ser Phe Val Val Asp Val Pro Asp Gly Asn Thr Arg Asp Glu Thr Cys Tyr Phe Val Glu Ala Leu Ile 150 155 Arg Cys Asn Leu Ser Ser Leu Ala Asp Val Ser Glu Arg Met Ala Val 170

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Gln Gly Arg Thr Asn Pro Ile Asn His
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<210> SEQ ID NO 130
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<213> ORGANISM: Glycine max
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<223> OTHER INFORMATION: soybean PYR/PYL receptor polypeptide
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Ile Cys Asp Gly Met Phe Cys Tyr Leu Val Asp Phe Val Asp Val Lys
Glu Lys Met Asn Tyr Cys Leu Met Trp Phe Gly Tyr Phe Pro Ser Gln
Val Trp Ser Leu Val Arg Arg Phe Asp Gln Pro Gln Lys Tyr Lys Pro
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Phe Val Ser Arg Cys Ile Met Gln Gly Asp Leu Gly Ile Gly Ser Val 65 70 75 80
Arg Glu Val Asn Val Lys Ser Gly Leu Pro Ala Thr Thr Ser Thr Glu
Arg Leu Glu Gln Leu Asp Asp Glu Glu His Ile Leu Gly Ile Arg Ile
Val Gly Gly Asp His Arg Leu Arg Asn Tyr Ser Ser Ile Ile Thr Val
                         120
His Pro Glu Val Ile Asp Gly Arg Pro Ser Thr Met Val Ile Glu Ser
                      135
Phe Val Val Asp Val Pro Asp Gly Asn Thr Arg Asp Glu Thr Cys Tyr
Phe Val Glu Ala Leu Ile Arg Cys Asn Leu Ser Ser Leu Ala Asp Val
                            170
Ser Glu Arg Met Ala Val Gln Gly Arg Thr Asp Pro Ile Asn His
<210> SEQ ID NO 131
<211> LENGTH: 185
<212> TYPE: PRT
<213 > ORGANISM: Glycine max
<220> FEATURE:
<223> OTHER INFORMATION: soybean PYR/PYL receptor polypeptide
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Arg His His Lys His Glu Pro Arg Glu Asn Gln Cys Thr Ser Ala Leu
Val Lys His Ile Arg Ala Pro Val His Leu Val Trp Ser Leu Val Arg
                          40
Arg Phe Asp Gln Pro Gln Lys Tyr Lys Pro Phe Val Ser Arg Cys Ile
           55
Met Gln Gly Asp Leu Gly Ile Gly Ser Val Arg Glu Val Asn Val Lys
Ser Gly Leu Pro Ala Thr Thr Ser Thr Glu Arg Leu Glu Gln Leu Asp
                                   90
Asp Glu Glu His Ile Leu Gly Ile Arg Ile Val Gly Gly Asp His Arg
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Leu Arg Asn Tyr Ser Ser Ile Ile Thr Val His Pro Glu Val Ile Asp
                         120
Gly Arg Pro Ser Thr Met Val Ile Glu Ser Phe Val Val Asp Val Pro
                      135
Asp Gly Asn Thr Arg Asp Glu Thr Cys Tyr Phe Val Glu Ala Leu Ile
Arg Cys Asn Leu Ser Ser Leu Ala Asp Val Ser Glu Arg Met Ala Val
                        170
Gln Gly Arg Thr Asp Pro Ile Asn His
<210> SEQ ID NO 132
<211> LENGTH: 204
<212> TYPE: PRT
<213 > ORGANISM: Sorghum bicolor
<220> FEATURE:
<223> OTHER INFORMATION: sorghum PYR/PYL receptor polypeptide
<400> SEQUENCE: 132
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Glu Val Arg Ala Leu Glu Pro Ala Val Arg Glu His His Thr Phe Pro
Ala Gly Arg Val Ala Ala Gly Thr Thr Thr Pro Thr Pro Thr Cys
Thr Ser Leu Val Ala Gln Arg Val Ser Ala Pro Val Arg Ala Val Trp
Pro Ile Val Arg Ser Phe Gly Asn Pro Gln Arg Tyr Lys His Phe Val
Arg Thr Cys Ala Leu Ala Ala Gly Asp Gly Ala Ser Val Gly Ser Val
Arg Glu Val Thr Val Val Ser Gly Leu Pro Ala Ser Ser Ser Thr Glu
Arg Leu Glu Val Leu Asp Asp Asp Arg His Ile Leu Ser Phe Arg Val
                           120
Val Gly Gly Asp His Arg Leu Arg Asn Tyr Arg Ser Val Thr Ser Val
Thr Glu Phe Gln Pro Gly Pro Tyr Cys Val Val Val Glu Ser Tyr Ala
Val Asp Val Pro Glu Gly Asn Thr Ala Glu Asp Thr Arg Met Phe Thr
Asp Thr Val Val Arg Leu Asn Leu Gln Lys Leu Ala Ala Val Ala Glu
Glu Ser Ala Ala Ala Ala Ala Gly Asn Arg Arg
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<210> SEQ ID NO 133
<211> LENGTH: 204
<212> TYPE: PRT
<213 > ORGANISM: Sorghum bicolor
<220> FEATURE:
<223> OTHER INFORMATION: sorghum PYR/PYL receptor polypeptide
<400> SEQUENCE: 133
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      5
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Leu Glu Gln Arg Glu Leu Glu Pro Val Val Arg Ala His His Thr Phe

199 200

25

20

Pro	Gly	Arg 35	Ser	Pro	Gly	Thr	Thr 40	Сув	Thr	Ser	Leu	Val 45	Thr	Gln	Arg
Val	Asp 50	Ala	Pro	Leu	Ser	Ala 55	Val	Trp	Pro	Ile	Val 60	Arg	Gly	Phe	Ala
Ala 65	Pro	Gln	Arg	Tyr	Lys 70	His	Phe	Ile	Lys	Ser 75	CÀa	Asp	Leu	Arg	Ser 80
Gly	Asp	Gly	Ala	Thr 85	Val	Gly	Ser	Val	Arg 90	Glu	Val	Thr	Val	Val 95	Ser
Gly	Leu	Pro	Ala 100	Ser	Thr	Ser	Thr	Glu 105	Arg	Leu	Glu	Ile	Leu 110	Asp	Asp
Asp	Arg	His 115	Ile	Leu	Ser	Phe	Arg 120	Val	Val	Gly	Gly	Asp 125	His	Arg	Leu
Arg	Asn 130	Tyr	Arg	Ser	Val	Thr 135	Ser	Val	Thr	Glu	Phe 140	His	His	His	His
Gln 145	Ala	Ala	Ala	Gly	Arg 150	Pro	Tyr	Cys	Val	Val 155	Val	Glu	Ser	Tyr	Val 160
Val	Asp	Val	Pro	Glu 165	Gly	Asn	Thr	Glu	Glu 170	Asp	Thr	Arg	Met	Phe 175	Thr
Asp	Thr	Val	Val 180	ГÀа	Leu	Asn	Leu	Gln 185	Lys	Leu	Ala	Ala	Ile 190	Ala	Thr
Ser	Ser	Ala 195	Ala	Ala	Ala	Ala	Ser 200	Asn	Ser	Ser	Thr				
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-continued

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                      25
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## What is claimed is:

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- 1. A plant comprising a heterologous expression cassette, the expression cassette comprising a drought-inducible or 30 tissue specific promoter operably linked to a polynucleotide encoding a PYR/PYL receptor polypeptide, wherein the PYR/PYL receptor polypeptide comprises SEQ ID NO:95 and further comprises SEQ ID NO:102 and is at least 95% identical to any of SEQ ID NOs:84-90, wherein the plant has 35 improved drought tolerance compared to a plant lacking the expression cassette.
- 2. The plant of claim 1, wherein the PYR/PYL receptor polypeptide is a constitutively-active form such that the receptor will bind a type 2 protein phosphatase (PP2C) in a 40 yeast two-hybrid assay in the absence of abscisic acid or an ABA agonist.
- 3. The plant of claim 1, wherein the PYR/PYL receptor polypeptide binds a type 2 protein phosphatase (PP2C) in a yeast two-hybrid assay in the presence, but not in the absence, 45 of abscisic acid or an ABA agonist.
- **4.** The plant of claim **1**, wherein the promoter is a drought-inducible promoter.
- **5**. A plant cell from the plant of claim **1** comprising the heterologous expression cassette.
- **6**. A seed, flower, leaf or fruit from the plant of claim **1** comprising the heterologous expression cassette.
- 7. An expression cassette comprising a heterologous drought-inducible or tissue specific promoter operably linked to a polynucleotide encoding a PYR/PYL receptor polypeptide, wherein the PYR/PYL receptor polypeptide comprises SEQ ID NO:95 and further comprises SEQ ID NO:102 and is at least 95% identical to any of SEQ ID NOs:84-90, wherein introduction of the expression cassette into a plant results in the plant having improved drought tolerance compared to a 60 plant lacking the expression cassette.
- **8**. The expression cassette of claim 7, wherein the PYR/PYL receptor polypeptide is a constitutively-active form such that the receptor will bind a type 2 protein phosphatase (PP2C) in a yeast two-hybrid assay in the absence of abscisic 65 acid.

**9**. The expression cassette of claim **7**, wherein the PYR/PYL receptor polypeptide binds a type 2 protein phosphatase (PP2C) in a yeast two-hybrid assay in the presence, but not in the absence, of abscisic acid.

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- 10. The expression cassette of claim 7, wherein the promoter is a drought-inducible promoter.
- 11. An expression vector comprising the expression cassette of claim 7.
- 12. A method of making a plant with increased drought tolerance, the method comprising
  - introducing the expression cassette of claim 7 into a plurality of plants; and
  - selecting a plant comprising the expression cassette having increased drought tolerance compared to a plant lacking the expression cassette.
- 13. The plant of claim 1, wherein the promoter is a guard cell-specific promoter.
- **14**. The expression cassette of claim **7**, wherein the promoter is a guard cell-specific promoter.
- **15**. The plant of claim **1**, wherein the PYR/PYL receptor polypeptide is at least 95% identical to SEQ ID NO:84.
- **16**. The plant of claim **1**, wherein the PYR/PYL receptor polypeptide is at least 95% identical to SEQ ID NO:85.
- 17. The plant of claim 1, wherein the PYR/PYL receptor polypeptide is at least 95% identical to SEQ ID NO:86.
- **18**. The plant of claim **1**, wherein the PYR/PYL receptor polypeptide is at least 95% identical to SEQ ID NO:87.
- 19. The plant of claim 1, wherein the PYR/PYL receptor polypeptide is at least 95% identical to SEQ ID NO:88.
- **20**. The plant of claim **1**, wherein the PYR/PYL receptor polypeptide is at least 95% identical to SEQ ID NO:89.
- 21. The plant of claim 1, wherein the PYR/PYL receptor polypeptide is at least 95% identical to SEQ ID NO:90.
- **22**. The plant of claim **1**, wherein the promoter is a shoot-specific, leaf-specific, or stem-specific promoter.
- 23. The expression cassette of claim 7, wherein the promoter is a shoot-specific, leaf-specific, or stem-specific promoter.

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